09/861,435

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=> d his

     (FILE 'HOME' ENTERED AT 10:21:50 ON 10 JUN 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:22:13 ON 10 JUN 2004
L1          1757 S BETA(W)GLYCOSIDASE?
L2         53153 S TETRAMER
L3            32 S L1 AND L2
L4             9 DUP REM L3 (23 DUPLICATES REMOVED)
L5          7080 S PYROCOCCUS
L6           156 S L1 AND L5
L7             8 S L2 AND L6
L8             3 DUP REM L7 (5 DUPLICATES REMOVED)
L9           741 S L5(A)HORIKOSHII
L10            6 S L1 AND L9
L11            2 DUP REM L10 (4 DUPLICATES REMOVED)
L12      6555393 S CLON? OR EXPRESS? OR RECOMBINANT
L13           75 S L6 AND L12
L14           26 DUP REM L13 (49 DUPLICATES REMOVED)
                 E KOSUGI Y/AU
L15          460 S E3
                 E ISHIDA H/AU
L16         6017 S E3
                 E ISHIKAWA K/AU
L17         8442 S E3
                 E MATSUI I/AU
L18          637 S E3
L19        15412 S L14 OR L15 OR L16 OR L17 OR L18
L20           29 S L1 AND L19
L21           26 DUP REM L20 (3 DUPLICATES REMOVED)
L22           26 S L21 AND L5
L23            2 S L9 AND L22
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NEWS	5	FEB 05	German (DE) application and patent publication number format changes
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=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci
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=> s beta(w)glycosidase?
L1 1757 BETA(W) GLYCOSIDASE?

=> s tetramer
L2 53153 TETRAMER

=> s l1 and l2
L3 32 L1 AND L2

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 9 DUP REM L3 (23 DUPLICATES REMOVED)

=> d 1-9 ibib ab

L4 ANSWER 1 OF 9 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2002684329 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12213823
TITLE: SDS-resistant active and thermostable dimers are obtained from the dissociation of homotetrameric **beta-glycosidase** from hyperthermophilic Sulfolobus solfataricus in SDS. Stabilizing role of the A-C intermonomeric interface.
AUTHOR: Gentile Fabrizio; Amodeo Pietro; Febbraio Ferdinando; Picaro Francesco; Motta Andrea; Formisano Silvestro; Nucci

Roberto
CORPORATE SOURCE: Istituto di Endocrinologia e Oncologia Sperimentale del CNR and Dipartimento di Biologia e Patologia Cellulare e Molecolare, Universita Federico II, Via Pansini 5, 80131 Napoli, Italy.
SOURCE: Journal of biological chemistry, (2002 Nov 15) 277 (46) 44050-60.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20021214
Last Updated on STN: 20030103
Entered Medline: 20030102

AB **beta-Glycosidases** are fundamental, widely conserved enzymes. Those from hyperthermophiles exhibit unusual stabilities toward various perturbants. Previous work with homotetrameric **beta-glycosidase** from hyperthermophilic Sulfolobus solfataricus (M(r) 226,760) has shown that addition of 0.05-0.1% SDS was associated with minimal secondary structure perturbations and increased activity. This work addresses the effects of SDS on **beta-glycosidase** quaternary structure. In 0.1-1% SDS, the enzyme was dimeric, as determined by Ferguson analysis of transverse-gradient polyacrylamide gels. The catalytic activity of the **beta-glycosidase** dimer in SDS was determined by in-gel assay. A minor decrease of thermal stability in SDS was observed after exposure to temperatures up to 80 degrees C for 1 h. An analysis of **beta-glycosidase** crystal structure showed different changes in solvent-accessible surface area on going from the **tetramer** to the two possible dimers (A-C and A-D). Energy minimization and molecular dynamics calculations showed that the A-C dimer, exhibiting the lowest exposed surface area, was more stabilized by a network of polar interactions. The charge distribution around the A-C interface was characterized by a local short range anisotropy, resulting in an unfavorable interaction with SDS. This paper provides a detailed description of an SDS-resistant inter-monomeric interface, which may help understand similar interfaces involved in important biological processes.

L4 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2000281794 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10819960
TITLE: Comparative structural analysis and substrate specificity engineering of the hyperthermostable beta-glucosidase CelB from Pyrococcus furiosus.
AUTHOR: Kaper T; Lebbink J H; Pouwels J; Kopp J; Schulz G E; van der Oost J; de Vos W M
CORPORATE SOURCE: Laboratory of Microbiology, Department of Biomolecular Sciences, Wageningen University, Hesselink van Suchtelenweg 4, NL-6703 CT Wageningen, The Netherlands.. thijs.kaper@algemeen.micr.wau.nl
SOURCE: Biochemistry, (2000 May 2) 39 (17) 4963-70.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000622
Last Updated on STN: 20020727
Entered Medline: 20000609

AB The substrate specificity of the beta-glucosidase (CelB) from the hyperthermophilic archaeon Pyrococcus furiosus, a family 1 glycosyl

hydrolase, has been studied at a molecular level. Following crystallization and X-ray diffraction of this enzyme, a 3.3 A resolution structural model has been obtained by molecular replacement. CelB shows a homo-**tetramer** configuration, with subunits having a typical (betaalpha)(8)-barrel fold. Its active site has been compared to the one of the previously determined 6-phospho-**beta-glycosidase** (LacG) from the mesophilic bacterium Lactococcus lactis. The overall design of the substrate binding pocket is very well conserved, with the exception of three residues that have been identified as a phosphate binding site in LacG. To verify the structural model and alter its substrate specificity, these three residues have been introduced at the corresponding positions in CelB (E417S, M424K, F426Y) in different combinations: single, double, and triple mutants. Characterization of the purified mutant CelB enzyme revealed that F426Y resulted in an increased affinity for galactosides, whereas M424K gave rise to a shifted pH optimum (from 5.0 to 6.0). Analysis of E417S revealed a 5-fold and a 3-fold increase of the efficiency of hydrolyzing o-nitrophenol-beta-D-galactopyranoside-6-phosphate, in the single and triple mutants, respectively. In contrast, their activity on nonphosphorylated sugars was largely reduced (30-300-fold). The residue at position E417 in CelB seems to be the determining factor for the difference in substrate specificity between the two types of family 1 glycosidases.

L4 ANSWER 3 OF 9 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 1999192316 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10094493
TITLE: Crystal structure of the **beta-glycosidase** from the hyperthermophile Thermosphaera aggregans: insights into its activity and thermostability.
AUTHOR: Chi Y I; Martinez-Cruz L A; Jancarik J; Swanson R V; Robertson D E; Kim S H
CORPORATE SOURCE: Department of Chemistry and Lawrence Berkeley National Laboratory, University of California, Berkeley 94720, USA.
SOURCE: FEBS letters, (1999 Feb 26) 445 (2-3) 375-83. Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF053078
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990504
Last Updated on STN: 19990504
Entered Medline: 19990420

AB The glycosyl hydrolases are an important group of enzymes that are responsible for cleaving a range of biologically significant carbohydrate compounds. Structural information on these enzymes has provided useful information on their molecular basis for the functional variations, while the characterization of the structural features that account for the high thermostability of proteins is of great scientific and biotechnological interest. To these ends we have determined the crystal structure of the **beta-glycosidase** from a hyperthermophilic archeon Thermosphaera aggregans. The structure is a (beta/alpha)8 barrel (TIM-barrel), as seen in other glycosyl hydrolase family 1 members, and forms a **tetramer**. Inspection of the active site and the surrounding area reveals two catalytic glutamate residues consistent with the retaining mechanism and the surrounding polar and aromatic residues consistent with a monosaccharide binding site. Comparison of this structure with its mesophilic counterparts implicates a variety of structural features that could contribute to the thermostability. These include an increased number of surface ion pairs, an increased number of internal water molecules and a decreased surface area upon forming an oligomeric quaternary structure.

L4 ANSWER 4 OF 9 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 97446327 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9299327
TITLE: Crystal structure of the **beta-glycosidase** from the hyperthermophilic archeon Sulfolobus solfataricus: resilience as a key factor in thermostability.
AUTHOR: Aguilar C F; Sanderson I; Moracci M; Ciaramella M; Nucci R; Rossi M; Pearl L H
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Univesity College London, Gower Street, London, WC1E 6BT, UK.
SOURCE: Journal of molecular biology, (1997 Sep 5) 271 (5) 789-802. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1GOW
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971021
Last Updated on STN: 19971021
Entered Medline: 19971008

AB Enzymes from hyperthermophilic organisms must operate at temperatures which rapidly denature proteins from mesophiles. The structural basis of this thermostability is still poorly understood. Towards a further understanding of hyperthermostability, we have determined the crystal structure of the **beta-glycosidase** (clan GH-1A, family 1) from the hyperthermophilic archaeon Sulfolobus solfataricus at 2.6 A resolution. The enzyme is a **tetramer** with subunit molecular mass at 60 kDa, and crystallises with half of the **tetramer** in the asymmetric unit. The structure is a (betaalpha)8 barrel, but with substantial elaborations between the beta-strands and alpha-helices in each repeat. The active site occurs at the centre of the top face of the barrel and is connected to the surface by a radial channel which becomes a blind-ended tunnel in the **tetramer**, and probably acts as the binding site for extended oligosaccharide substrates. Analysis of the structure reveals two features which differ significantly from mesophile proteins; (1) an unusually large proportion of surface ion-pairs involved in networks that cross-link sequentially separate structures on the protein surface, and (2) an unusually large number of solvent molecules buried in hydrophilic cavities between sequentially separate structures in the protein core. These factors suggest a model for hyperthermostability via resilience rather than rigidity.

L4 ANSWER 5 OF 9 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1995-07320 BIOTECHDS
TITLE: Thermostable **beta-glycosidase** from Sulfolobus solfataricus; enzyme characterization
AUTHOR: Moracci M; Ciaramella M; Nucci R; Pearl L H; Sanderson I; Trincone A; *Rossi M
CORPORATE SOURCE: Inst.Protein-Biochem.Enzymol.Naples; Inst.Chem.Mol.Biol.Naples; Univ.London
LOCATION: Institute of Protein Biochemistry and Enzymology, Via Marconi, 10, 80125, Naples, Italy.
SOURCE: Biocatalysis; (1995) 11, 2, 89-103
CODEN: BIOCED
ISSN: 0886-4454
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The Sulfolobus solfataricus MT-4 **beta-glycosidase** is a thermostable and thermophilic glycosyl-hydrolase with broad substrate specificity. The enzyme was found to be a **tetramer** of mol.weight 240,000 (60,000 subunit mol.weight). The enzyme was activated by heat

showing enhanced activity up to 95 deg. It was also highly resistant to proteases and surfactants. The enzyme hydrolyzed beta-D-gluco-, fuco- and galactosides and a large number of beta-linked glycoside dimers and oligomers, linked beta-1-3, beta-1-4 and beta-1-6. The S. solfataricus hydrolyzed oligosaccharides with up to 5 glucose residues and promoted transglycosylation reactions. The corresponding gene was cloned and overexpressed in yeast and Escherichia coli. Based on sequence and functional data, the S. solfataricus **beta-glycosidase** was assigned to the BGA family of glycosyl-hydrolases, including **beta-glycosidases**, beta-galactosidases and phospho-beta-galactosidases from mesophilic and thermophilic organisms of the 3 domains. The S. solfataricus enzyme was crystallized and the resolution of its structure was initiated. The S. solfataricus enzyme has biotechnological potential. (21 ref)

L4 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:11578 HCAPLUS
DOCUMENT NUMBER: 124:80132
TITLE: Glycosidases, large and small: T4 lysozyme and Escherichia coli β-galactosidase
AUTHOR(S): Jacobson, R. H.; Kuroki, R.; Weaver, L. H.; Zhang, X.-J.; Matthews, B. W.
CORPORATE SOURCE: Inst. of Molecular Biology, Univ. of Oregon, Eugene, OR, 97403, USA
SOURCE: ACS Symposium Series (1995), 618(Enzymatic Degradation of Insoluble Carbohydrates), 38-50
CODEN: ACSMC8; ISSN: 0097-6156
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review, with 33 refs. T4 lysozyme (TL4) and E. coli β-galactosidase provide contrasting examples of glycosidases. Their three-dimensional structures are shown to be substantially different, not only with regard to size, but in other respects as well. β-Galactosidase is a **tetramer** of four identical subunits. Within each subunit the 1023-amino acid polypeptide chain folds into five sequential domains plus an extended segment at the amino terminus. Each of the four active sites in the **tetramer** is formed by elements from two different subunits. In contrast, T4L is a monomeric protein of 164 amino acids with the active site at the junction of the amino-terminal and carboxy-terminal domains. The mutation Thr 26→Glu in the active site cleft of phage T4 lysozyme produces an enzyme that cleaves the cell wall of Escherichia coli but leaves the product covalently bound to the enzyme. The crystalline complex is non-isomorphous with wild-type T4L and anal. of its structure shows a covalent linkage between the product and the newly-introduced Glu 26. The covalently-linked sugar ring is substantially distorted, suggesting that distortion of the substrate toward the transition state is important for catalysis, as originally proposed by Phillips. It is also postulated that the adduct formed by the mutant is an intermediate consistent with a double displacement mechanism of action (in the mutant) in which the glycosidic linkage is cleaved with retention of configuration as originally proposed by Koshland. The lysozymes and **.beta.-glycosidases** provide contrasting examples of glycosidases. Hen egg-white lysozyme, the prototypical lysozyme, is a small monomeric enzyme of 129 amino acids. In contrast, the **.beta.-glycosidase** from Escherichia coli is a larger tetrameric enzyme (MW = 465,412 Da). Here, the recently determined structure of **.beta.-glycosidase** is described and contrasted with the much small enzyme, bacteriophage T4 lysozyme.

L4 ANSWER 7 OF 9 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 89321548 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2751308
TITLE: Inhibition of rabbit muscle glycogen phosphorylase by

D-gluconohydroximo-1,5-lactone-N-phenylurethane.
AUTHOR: Papageorgiou A C; Oikonomakos N G; Leonidas D D
CORPORATE SOURCE: National Hellenic Research Foundation, Athens, Greece.
SOURCE: Archives of biochemistry and biophysics, (1989 Aug 1) 272 (2) 376-85.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198908
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19980206
Entered Medline: 19890816

AB The effect of the **beta-glycosidase** inhibitor D-gluconohydroximo-1,5-lactone-N-phenylurethane (PUG) on the kinetic and ultracentrifugation properties of glycogen phosphorylase has been studied. Recent crystallographic work at 2.4 A resolution [D. Barford et al. (1988) Biochemistry 27, 6733-6741] has shown that PUG binds in the catalytic site of phosphorylase b crystals with its gluconohydroximolactone moiety occupying a position similar to that observed for other glucosyl compounds and the N-phenylurethane side chain fitting into an adjacent cavity with little conformational change in the enzyme. In solution, PUG was shown to be a potent inhibitor of phosphorylase b, directly competitive with alpha-D-glucopyranose 1-phosphate (glucose-1-P) (Ki = 0.40 mM) and noncompetitive with respect to glycogen and AMP. When PUG was tested for synergistic inhibition in the presence of caffeine, the Dixon plots of reciprocal velocity versus PUG concentration at different fixed caffeine concentrations provided intersecting lines with interaction constant (alpha) values of 0.95-1.38, indicating that the binding of one inhibitor is not significantly affected by the binding of the other. For glycogen phosphorolysis, PUG was noncompetitive with respect to phosphate, suggesting that it can bind to the central enzyme-AMP-glycogen-phosphate complex. PUG was shown to inhibit phosphorylase alpha (without AMP) activity (Ki = 0.43 mM) in a manner similar to that of the b form. However, in the presence of AMP, PUG exhibited complex kinetics, acting as a noncompetitive inhibitor with respect to glucose-1-P, while a twofold decrease of PUG binding to the enzyme-AMP-glycogen complex was observed. Ultracentrifugation experiments demonstrated that PUG does not cause any significant dissociation of phosphorylase alpha **tetramer**. Furthermore the dimerization of phosphorylase alpha by glucose is completely prevented in the presence of PUG. These observations are consistent with PUG binding to both the R and the T conformations of phosphorylase.

L4 ANSWER 8 OF 9 MEDLINE on STN
ACCESSION NUMBER: 87126773 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3101594
TITLE: Characterization of beta-glucosidases with high specificity for the cyanogenic glucoside dhurrin in Sorghum bicolor (L.) moench seedlings.
AUTHOR: Hosel W; Tober I; Eklund S H; Conn E E
CONTRACT NUMBER: GM-05301-27 (NIGMS)
SOURCE: Archives of biochemistry and biophysics, (1987 Jan) 252 (1) 152-62.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198703
ENTRY DATE: Entered STN: 19900303
Last Updated on STN: 19970203
Entered Medline: 19870304

AB Two beta-glucosidases exhibiting high specificity for the cyanogenic glucoside dhurrin have been purified to near homogeneity from seedlings of Sorghum bicolor. Dhurrinase 1 was isolated from shoots of seedlings grown in the dark. Dhurrinase 2 was isolated from the green shoots of young seedlings grown in the light. The two enzymes were similar in following characteristics: their optimum activity is around pH 6.2; the enzymes are stable above pH 7; they are effectively inhibited by the **beta-glycosidase** inhibitors nojirimycin delta-gluconolactone and 1-amino-beta-D-glucoside. On the other hand, they clearly differed in other properties, e.g., molecular weights, isoelectric points, and substrate specificity. Moreover, dithiothreitol has no effect on dhurrinase 1, but is necessary for the activity of dhurrinase 2. Preliminary investigations indicate that the two enzymes are located in different parts of the sorghum seedlings: dhurrinase 1 is found in the coleoptiles and hypocotyls; dhurrinase 2 occurs in the leaves. Dhurrin (p-hydroxy-(S)-mandelonitrile-beta-D-glucoside) and its structural analog without the hydroxyl group, sambunigrin, were the only substrates hydrolyzed at high rate, the Km values with both enzymes being 0.15 and 0.3 mM, respectively. All other cyanogenic glucosides tested, as well as synthetic substrates such as 4-nitrophenyl-beta-D-glucoside, were in general poor substrates, especially for dhurrinase 1, the enzyme isolated from coleoptile and hypocotyl tissue. Dhurrinase 1 appears to exist within the seedlings as a **tetramer** (Mr - 2-2.4 X 10(5)) which dissociates without loss of activity into a dimeric form (Mr = 1-1.1 X 10(5)) upon extraction and purification. There is only one monomeric subunit with Mr = 5.7 X 10(4). Isolectric focusing and chromatofocusing of purified dhurrinase 1 showed the presence of at least three isomeric forms, but their relationship to each other is not known at the present time. Dhurrinase 2 appears to be a tetrameric protein with Mr = 2.5-3 X 10(5); it also has only one monomeric subunit of Mr = 6.1 X 10(4). In contrast to many other beta-glucosidases, the dhurrinases are not glycoproteins.

L4 ANSWER 9 OF 9 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1984:222030 BIOSIS
DOCUMENT NUMBER: PREV198477055014; BA77:55014
TITLE: PARTIAL CHARACTERIZATION OF A THERMOSTABLE ANTHO CYANIN **BETA GLYCOSIDASE** FROM ASPERGILLUS-NIGER.
AUTHOR(S): BLOM H [Reprint author]
CORPORATE SOURCE: NORWEGIAN FOOD RES INST, PO BOX 50, N-1432 AAS-NLH, NORWAY
SOURCE: Food Chemistry, (1983) Vol. 12, No. 3, pp. 197-204.
CODEN: FOCHDJ. ISSN: 0308-8146.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB Anthocyanin-.**beta**.-**glycosidase** (anthocyanase) from A. niger was partly purified and catalyzes a lytic reaction upon the β-glycosidic bond of anthocyanins. The MW as estimated from high performance liquid chromatography and gel chromatography on Ultrogel AcA-34 is in the range (370 ± 30) + 103. Sodium dodecyl sulfate electrophoresis indicates a tetrameric composition; the isoelectric point is located at pH 4.

=> d his

(FILE 'HOME' ENTERED AT 10:21:50 ON 10 JUN 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:22:13 ON 10 JUN 2004
L1 1757 S BETA(W)GLYCOSIDASE?
L2 53153 S TETRAMER
L3 32 S L1 AND L2
L4 9 DUP REM L3 (23 DUPLICATES REMOVED)

```
=> s pyrococcus
L5        7080 PYROCOCCUS

=> s l1 and l5
L6         156 L1 AND L5

=> s l2 and l6
L7           8 L2 AND L6

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8           3 DUP REM L7 (5 DUPLICATES REMOVED)

=> d 1-3 ibib ab

L8   ANSWER 1 OF 3     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:   2000281794     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 10819960
TITLE:              Comparative structural analysis and substrate specificity
                    engineering of the hyperthermostable beta-glucosidase CelB
                    from Pyrococcus furiosus.
AUTHOR:             Kaper T; Lebbink J H; Pouwels J; Kopp J; Schulz G E; van
                    der Oost J; de Vos W M
CORPORATE SOURCE:   Laboratory of Microbiology, Department of Biomolecular
                    Sciences, Wageningen University, Hesselink van Suchtelenweg
                    4, NL-6703 CT Wageningen, The Netherlands..
                    thijs.kaper@algemeen.micr.wau.nl
SOURCE:             Biochemistry, (2000 May 2) 39 (17) 4963-70.
                    Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200006
ENTRY DATE:         Entered STN: 20000622
                    Last Updated on STN: 20020727
                    Entered Medline: 20000609
AB   The substrate specificity of the beta-glucosidase (CelB) from the
     hyperthermophilic archaeon Pyrococcus furiosus, a family 1
     glycosyl hydrolase, has been studied at a molecular level. Following
     crystallization and X-ray diffraction of this enzyme, a 3.3 A resolution
     structural model has been obtained by molecular replacement. CelB shows a
     homo-tetramer configuration, with subunits having a typical
     (betaalpha)(8)-barrel fold. Its active site has been compared to the one
     of the previously determined 6-phospho-beta-glycosidase
     (LacG) from the mesophilic bacterium Lactococcus lactis. The overall
     design of the substrate binding pocket is very well conserved, with the
     exception of three residues that have been identified as a phosphate
     binding site in LacG. To verify the structural model and alter its
     substrate specificity, these three residues have been introduced at the
     corresponding positions in CelB (E417S, M424K, F426Y) in different
     combinations: single, double, and triple mutants. Characterization of the
     purified mutant CelB enzyme revealed that F426Y resulted in an increased
     affinity for galactosides, whereas M424K gave rise to a shifted pH optimum
     (from 5.0 to 6.0). Analysis of E417S revealed a 5-fold and a 3-fold
     increase of the efficiency of hydrolyzing o-nitrophenol-beta-D-
     galactopyranoside-6-phosphate, in the single and triple mutants,
     respectively. In contrast, their activity on nonphosphorylated sugars was
     largely reduced (30-300-fold). The residue at position E417 in CelB seems
     to be the determining factor for the difference in substrate specificity
     between the two types of family 1 glycosidases.

L8   ANSWER 2 OF 3  SCISEARCH  COPYRIGHT 2004 THOMSON ISI on STN
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ACCESSION NUMBER: 1999:224240 SCISEARCH
THE GENUINE ARTICLE: 175YC
TITLE: Crystal structure of the **beta-glycosidase** from the hyperthermophile Thermosphaera aggregans: insights into its activity and thermostability
AUTHOR: Chi Y I; MartinezCruz L A; Jancarik J; Swanson R V; Robertson D E; Kim S H (Reprint)
CORPORATE SOURCE: UNIV CALIF BERKELEY, DEPT CHEM, BERKELEY, CA 94720 (Reprint); UNIV CALIF BERKELEY, DEPT CHEM, BERKELEY, CA 94720; UNIV CALIF BERKELEY, LAWRENCE BERKELEY LAB, BERKELEY, CA 94720; DIVERSA CORP, SAN DIEGO, CA 92121
COUNTRY OF AUTHOR: USA
SOURCE: FEBS LETTERS, (26 FEB 1999) Vol. 445, No. 2-3, pp. 375-383 .
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0014-5793.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 43
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The glycosyl hydrolases are an important group of enzymes that are responsible for cleaving a range of biologically significant carbohydrate compounds. Structural information on these enzymes has provided useful information on their molecular basis for the functional variations, while the characterization of the structural features that account for the high thermostability of proteins is of great scientific and biotechnological interest. To these ends we have determined the crystal structure of the **beta-glycosidase** from a hyperthermophilic archeon Thermosphaera a aggregans. The structure is a (beta/alpha)(8) barrel (TIM-barrel), as seen in other glycosyl hydrolase family 1 members, and forms a **tetramer**. Inspection of the active site and the surrounding area reveals two catalytic glutamate residues consistent with the retaining mechanism and the surrounding polar and aromatic residues consistent with a monosaccharide binding site. Comparison of this structure with its mesophilic counterparts implicates a variety of structural features that could contribute to the thermostability. These include an increased number of surface ion pairs, an increased number of internal water molecules and a decreased surface area upon forming an oligomeric quaternary structure. (C) 1999 Federation of European Biochemical Societies.

L8 ANSWER 3 OF 3 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 97:683656 SCISEARCH
THE GENUINE ARTICLE: XU907
TITLE: Crystal structure of the **beta-glycosidase** from the hyperthermophilic archeon Sulfolobus solfataricus: Resilience as a key factor in thermostability
AUTHOR: Aguilar C F; Sanderson I; Moracci M; Ciaramella M; Nucci R; Rossi M; Pearl L H (Reprint)
CORPORATE SOURCE: UNIV LONDON UNIV COLL, DEPT BIOCHEM & MOL BIOL, STRUCT BIOCHEM SECT, GOWER ST, LONDON WC1E 6BT, ENGLAND (Reprint); UNIV LONDON UNIV COLL, DEPT BIOCHEM & MOL BIOL, STRUCT BIOCHEM SECT, LONDON WC1E 6BT, ENGLAND; CNR, IST BIOCHIM PROT & ENZIMOL, I-80125 NAPLES, ITALY
COUNTRY OF AUTHOR: ENGLAND; ITALY
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (5 SEP 1997) Vol. 271, No. 5, pp. 789-802.
Publisher: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON, ENGLAND NW1 7DX.
ISSN: 0022-2836.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 49

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Enzymes from hyperthermophilic organisms must operate at temperatures which rapidly denature proteins from mesophiles. The structural basis of this thermostability is still poorly understood. Towards a further understanding of hyperthermostability we have determined the crystal structure of the **beta-glycosidase** (dan GH-1A, family 1) from the hyperthermophilic archaeon Sulfolobus solfataricus at 2.6 Angstrom resolution. The enzyme is a **tetramer** with subunit molecular mass at 60 kDa, and crystallises with half of the **tetramer** in the asymmetric unit. The structure is a (beta alpha)(8) barrel, but with substantial elaborations between the beta-strands and alpha-helices in each repeat. nle active site occurs at the centre of the top face of the barrel and is connected to the surface by a radial channel which becomes a blind-ended tunnel in the **tetramer**, and probably acts as the binding site for extended oligosaccharide substrates. Analysis of the structure reveals two features which differ significantly from mesophile proteins; (1) an unusually large proportion of surface ion-pairs involved in networks that cross-link sequentially separate structures on the protein surface, and (2) an unusually large number of solvent molecules buried in hydrophilic cavities between sequentially separate structures in the protein core. These factors suggest a model for hyperthermostability via resilience rather than rigidity. (C) 1997 Academic Press Limited.

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=> d his

     (FILE 'HOME' ENTERED AT 10:21:50 ON 10 JUN 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:22:13 ON 10 JUN 2004
L1          1757 S BETA(W)GLYCOSIDASE?
L2         53153 S TETRAMER
L3            32 S L1 AND L2
L4             9 DUP REM L3 (23 DUPLICATES REMOVED)
L5          7080 S PYROCOCCUS
L6           156 S L1 AND L5
L7             8 S L2 AND L6
L8             3 DUP REM L7 (5 DUPLICATES REMOVED)

=> s l5(a)horikoshii
L9           741 L5(A) HORIKOSHII

=> s l1 and l9
L10            6 L1 AND L9

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11            2 DUP REM L10 (4 DUPLICATES REMOVED)

=> d 1-2 ibib ab

L11  ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2000:121661  HCAPLUS
DOCUMENT NUMBER:          132:177431
TITLE:                    Preparation of a heat-resistant .beta.-
                          glycosidase of Pyrococcus
                          horikoshii and cloning of its encoding gene
INVENTOR(S):              Matsui, Ikuo; Ishikawa, Kazuhiko; Ishida, Hiroyasu;
                          Kosugi, Keiji
```

PATENT ASSIGNEE(S): Agency of Industrial Sciences and Technology, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2000050870	A2	20000222	JP 1998-222866	19980806
JP 2995292	B2	19991227		
US 2002102635	A1	20020801	US 1999-369735	19990806

PRIORITY APPLN. INFO.: JP 1998-222866 A 19980806

AB The gene encoding a novel heat-resistant .**beta**.-**glycosidase** is isolated from **Pyrococcus horikoshii** strain JCM9974. The .**beta**.-**glycosidase** prepared from transgenic Escherichia coli exhibits a temperature optimum >100° and a pH optimum 6.0.

L11 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2000141228 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10675537
TITLE: Novel substrate specificity of a membrane-bound **beta-glycosidase** from the hyperthermophilic archaeon **Pyrococcus horikoshii**.
AUTHOR: Matsui I; Sakai Y; Matsui E; Kikuchi H; Kawarabayasi Y; Honda K
CORPORATE SOURCE: National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki, Japan.. ikmatsui@nibh.go.jp
SOURCE: FEBS letters, (2000 Feb 11) 467 (2-3) 195-200.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000413
Last Updated on STN: 20000413
Entered Medline: 20000331

AB A **beta-glycosidase** gene homolog of **Pyrococcus horikoshii** (BGPh) was successfully expressed in Escherichia coli. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85 degrees C for 15 min. The optimum pH was 6.0 and the optimum temperature was over 100 degrees C, respectively. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90 degrees C (pH 6.0) was 15 h. BGPh has a novel substrate specificity with k(cat)/K(m) values high enough for hydrolysis of beta-D-Glcp derivatives with long alkyl chain at the reducing end and low enough for the hydrolysis of beta-linked glucose dimer more hydrophilic than aryl- or alkyl-beta-D-Glcp.

=> d his

(FILE 'HOME' ENTERED AT 10:21:50 ON 10 JUN 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:22:13 ON 10 JUN 2004

L1 1757 S BETA(W)GLYCOSIDASE?
L2 53153 S TETRAMER
L3 32 S L1 AND L2
L4 9 DUP REM L3 (23 DUPLICATES REMOVED)

```
L5          7080 S PYROCOCCUS
L6           156 S L1 AND L5
L7             8 S L2 AND L6
L8             3 DUP REM L7 (5 DUPLICATES REMOVED)
L9           741 S L5(A)HORIKOSHII
L10            6 S L1 AND L9
L11            2 DUP REM L10 (4 DUPLICATES REMOVED)

=> s clon? or express? or recombinant
   5 FILES SEARCHED...
L12    6555393 CLON? OR EXPRESS? OR RECOMBINANT

=> s l6 and l12
L13          75 L6 AND L12

=> dup rem l13
PROCESSING COMPLETED FOR L13
L14           26 DUP REM L13 (49 DUPLICATES REMOVED)

=> d 1-26 ibib ab

L14  ANSWER 1 OF 26     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:   2003361724     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 12859194
TITLE:              Activity of hyperthermophilic glycosynthases is
                    significantly enhanced at acidic pH.
AUTHOR:             Perugino Giuseppe; Trincone Antonio; Giordano Assunta; van
                    der Oost John; Kaper Thijs; Rossi Mose; Moracci Marco
CORPORATE SOURCE:   Institute of Protein Biochemistry-Consiglio Nazionale delle
                    Ricerche, Via P. Castellino 111, 80131, Naples Italy.
SOURCE:             Biochemistry, (2003 Jul 22) 42 (28) 8484-93.
                    Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200310
ENTRY DATE:         Entered STN: 20030805
                    Last Updated on STN: 20031008
                    Entered Medline: 20031006
AB   We have previously shown that the hyperthermophilic glycosynthase from
     Sulfolobus solfataricus (Ssbeta-glyE387G) can promote the synthesis of
     branched oligosaccharides from activated beta-glycosides, at pH 6.5, in
     the presence of 2 M sodium formate as an external nucleophile. In an
     effort to increase the synthetic potential of hyperthermophilic
     glycosynthases, we report a new method to reactivate the Ssbeta-glyE387G
     glycosynthase and two novel mutants in the nucleophile of the beta
     -glycosidases from the hyperthermophilic Archaea Thermosphaera
     aggregans (Tabeta-gly) and Pyrococcus furiosus (CelB). We
     describe here that, at pH 3.0 and low concentrations of sodium formate
     buffer, the three hyperthermophilic glycosynthases show k(cat) values
     similar to those of the wild-type enzymes and 17-fold higher than those
     observed at the usual reactivation conditions in 2 M sodium formate at pH
     6.5. Moreover, at acidic pH the three reactivated mutants have wide
     substrate specificity and improved efficiency in the synthetic reaction.
     The data reported suggest that the reactivation conditions modify the
     ionization state of the residue acting as an acid/base catalyst. This new
     reactivation method can be of general applicability on hyperthermophilic
     glycosynthases whose intrinsic stability allows their exploitation as
     synthetic tools at low pH.

L14  ANSWER 2 OF 26  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
     DUPLICATE 2
ACCESSION NUMBER:   2002:563403  BIOSIS
```

DOCUMENT NUMBER: PREV200200563403
TITLE: The temperature influences the ratio of glucosidase and galactosidase activities of **beta-glycosidases**.
AUTHOR(S): Hansson, Therese; Adlercreutz, Patrick [Reprint author]
CORPORATE SOURCE: Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, SE-221 00, P.O. Box 124, Lund, Sweden
patrick.adlercreutz@biotek.lu.se
SOURCE: Biotechnology Letters, (September, 2002) Vol. 24, No. 18, pp. 1465-1471. print.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Oct 2002
Last Updated on STN: 30 Oct 2002

AB The selectivity for the glycon part of a donor substrate of **beta-glycosidases** from almond, a mesophilic (Kluyveromyces fragilis) and three highly thermophilic organisms (Caldocellum saccharolyticum, Sulfolobus solfataricus and **Pyrococcus** furiosus) was investigated at various temperatures (25-90 degreeC). On the basis of kinetic constants, the selectivity was calculated as the specificity constant (Vmax/Km) ratio or Vmax ratio of glucoside to galactoside donor. In the almond beta-glucosidase and the mesostable enzyme one enzyme activity dominated whereas the thermostable enzymes **expressed** both high beta-glucosidase and high beta-galactosidase activities. Surprisingly, for **beta-glycosidases** from almond, K. fragilis, and C. saccharolyticum the donor selectivity decreased as the temperature increased. In contrast, two of the highly thermostable enzymes (from S. solfataricus and P. furiosus) had constant donor selectivity as the temperature increased. The results thus showed **beta-glycosidases** of differing origins to differ markedly in their substrate specificity and in the extent to which their selectivity for the glycon part of the donor substrate is influenced by the temperature.

L14 ANSWER 3 OF 26 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2002080570 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11807757
TITLE: Development of an ultrahigh-temperature process for the enzymatic hydrolysis of lactose. IV. Immobilization of two thermostable **beta-glycosidases** and optimization of a packed-bed reactor for lactose conversion.
AUTHOR: Petzelbauer Inge; Kuhn Bernhard; Splechtna Barbara; Kulbe Klaus D; Nidetzky Bernd
CORPORATE SOURCE: Division of Biochemical Engineering, Institute of Food Technology, Universitat fur Bodenkultur Wien (BOKU), Muthgasse 18, A-1190 Vienna, Austria.
SOURCE: Biotechnology and bioengineering, (2002 Mar 20) 77 (6) 619-31.
Journal code: 7502021. ISSN: 0006-3592.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020128
Last Updated on STN: 20020518
Entered Medline: 20020517

AB **Recombinant** hyperthermostable **beta-glycosidases** from the archaea Sulfolobus solfataricus (Ss beta Gly) and **Pyrococcus** furiosus (CelB) were covalently attached onto the insoluble carriers chitosan, controlled pore glass (CPG), and

Eupergit C. For each enzyme/carrier pair, the protein-binding capacity, the immobilization yield, the pH profiles for activity and stability, the activity/temperature profile, and the kinetic constants for lactose hydrolysis at 70 degrees C were determined. Eupergit C was best among the carriers in regard to retention of native-like activity and stability of Ss beta Gly and CelB over the pH range 3.0-7.5. Its protein binding capacity of approximately 0.003 (on a mass basis) was one-third times that of CPG, while immobilization yields were typically 80% in each case. Activation energies for lactose conversion by the immobilized enzymes at pH 5.5 were in the range 50-60 kJ/mol. This is compared to values of approximately 75 kJ/mol for the free enzymes. Immobilization expands the useful pH range for CelB and Ss beta Gly by approximately 1.5 pH units toward pH 3.5 and pH 4.5, respectively. A packed-bed enzyme reactor was developed for the continuous conversion of lactose in different media, including whey and milk, and operated over extended reaction times of up to 14 days. The productivities of the Eupergit C-immobilized enzyme reactor were determined at dilution rates between 1 and 12 h(-1), and using 45 and 170 g/L initial lactose. Results of kinetic modeling for the same reactor, assuming plug flow and steady state, suggest the presence of mass-transfer limitation of the reaction rate under the conditions used. Formation of galacto-oligosaccharides in the continuous packed-bed reactor and in the batch reactor using free enzyme was closely similar in regard to yield and individual saccharide components produced.
 Biotechnol Bioeng 77: 619-631, 2002; DOI 10.1002/bit.10110

L14 ANSWER 4 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:119696 SCISEARCH
THE GENUINE ARTICLE: 517AH
TITLE: Regulation of endo-acting glycosyl hydrolases in the hyperthermophilic bacterium Thermotoga maritima grown on glucan- and mannan-based polysaccharides
AUTHOR: Chhabra S R; Shockley K R; Ward D E; Kelly R M (Reprint)
CORPORATE SOURCE: N Carolina State Univ, Dept Chem Engn, Stinson Dr, Box 7905, Raleigh, NC 27695 USA (Reprint); N Carolina State Univ, Dept Chem Engn, Raleigh, NC 27695 USA
COUNTRY OF AUTHOR: USA
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (FEB 2002) Vol. 68, No. 2, pp. 545-554.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0099-2240.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 56

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The genome sequence of the hyperthermophilic bacterium Thermotoga maritima encodes a number of glycosyl hydrolases. Many of these enzymes have been shown in vitro to degrade specific glycosides that presumably serve as carbon and energy sources for the organism. However, because of the broad substrate specificity of many glycosyl hydrolases, it is difficult to determine the physiological substrate preferences for specific enzymes from biochemical information. In this study, T. maritima was grown on a range of polysaccharides, including barley beta-glucan, carboxymethyl cellulose, carob galactomannan, konjac glucomannan, and potato starch. In all cases, significant growth was observed, and cell densities reached 109 cells/ml. Northern blot analyses revealed different substrate-dependent **expression** patterns for genes encoding the various endo-acting **beta-glycosidases**; these patterns ranged from strong **expression** to no **expression** under the conditions tested. For example, cel74 (TM0305), a gene encoding a putative beta-specific endoglucananse, was strongly **expressed** on all substrates tested, including starch, while no evidence of **expression** was observed on any substrate for lam16 (TM0024),

xyl10A (TM0061), xyl10B (TM0070), and cel12A (TM1524), which are genes that encode a laminarinase, two xylanases, and an endoglucanase, respectively. The cel12B (TM1525) gene, which encodes an endoglucanase, was **expressed** only on carboxymethyl cellulose. An extracellular mannanase encoded by man5 (TM1227) was **expressed** on carob galactomannan and konjac glucomannan and to a lesser extent on carboxymethyl cellulose. An unexpected result was the finding that the cel5.4 (TM1751) and cel5B (TM1752) genes, which encode putative intracellular, beta-specific endoglucanases, were induced only when T. maritima was grown on konjac glucomannan. To investigate the biochemical basis of this finding, the **recombinant** forms of Man5 (M-r, 76,900) and Cel5A (M-r, 37,400) were **expressed** in Escherichia coli and characterized. Man5, a T. maritima extracellular enzyme, had a melting temperature of 99degreesC and an optimun temperature of 90degreesC, compared to 90 and 80degreesC, respectively, for the intracellular enzyme Cel5A. While Man5 hydrolyzed both galactomannan and glucomannan, no activity was detected on glucans or xylans. Cel5A, however, not only hydrolyzed barley P-glucan, carboxymethyl cellulose, xyloglucan, and lichenin but also had activity comparable to that of Man5 on galactomannan and higher activity than Man5 on glucomannan. The biochemical characteristics of Cel5A, the fact that Cel5A was induced only when T. maritima was grown on glucomannan, and the intracellular localization of Cel5A suggest that the physiological role of this enzyme includes hydrolysis of glucomannan oligosaccharides that are transported following initial hydrolysis by extracellular glycosidases, such as Man5.

L14 ANSWER 5 OF 26 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 2002277402 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12018273
TITLE: Hydrolysis of lactose by **beta-glycosidase** CelB from hyperthermophilic archaeon **Pyrococcus** furiosus: comparison of hollow-fiber membrane and packed-bed immobilized enzyme reactors for continuous processing of ultrahigh temperature-treated skim milk.
AUTHOR: Splechtna Barbara; Petzelbauer Inge; Kuhn Bernhard; Kulbe Klaus D; Nidetzky Bernd
CORPORATE SOURCE: Division of Biochemical Engineering, Institute of Food Technology, University of Agricultural Sciences, Vienna, Austria.
SOURCE: Applied biochemistry and biotechnology, (2002 Spring) 98-100 473-88.
Journal code: 8208561. ISSN: 0273-2289.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20020522
Last Updated on STN: 20021211
Entered Medline: 20021106

AB **Recombinant beta-glycosidase** CelB from the hyperthermophilic archaeon Pyrococcusfuriosus was produced through **expression** of the plasmid-encoded gene in Escherichia coli. Bioreactor cultivations of E. coli in the presence of the inductor isopropyl-1-thio-beta-D-galactoside (0.1 mM) gave approx 100,000 U of enzyme activity/L of culture medium after 8 h of growth. A technical-grade enzyme for the hydrolysis of lactose was prepared by precipitating the mesophilic protein at 80 degrees C. A hollow-fiber membrane reactor was developed, and its performance during continuous processing of ultrahigh temperature-treated (UHT) skim milk at 70 degrees C was analyzed regarding long-term stability, productivity, and diffusional limitation thereof. CelB was covalently attached onto Eupergit C in yields of 80%, and a packed-bed immobilized enzyme reactor was used for the continuous hydrolysis of lactose in UHT skim milk at 70

degrees C. The packed-bed reactor was approximately 10-fold more stable and gave about the same productivity at 80% substrate conversion as the hollow-fiber reactor at 60% substrate conversion. The marked difference in the stability of free and immobilized CelB seems to reflect mainly binding of the soluble enzyme to the membrane surface of the hollow-fiber module. Under these bound conditions, CelB is essentially inactive. CelB is essentially inactive. Microbial contamination of the reactors did not occur during reaction times of up to 39 d, given that UHT skim milk and not pasteurized skim milk was used as the substrate.

L14 ANSWER 6 OF 26 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2002680611 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12164784
TITLE: DNA family shuffling of hyperthermostable **beta-glycosidases**.
AUTHOR: Kaper Thijs; Brouns Stan J J; Geerling Ans C M; De Vos Willem M; Van der Oost John
CORPORATE SOURCE: Laboratory of Microbiology, Wageningen University, Hessenlink van Suchtelenweg 4, NL-6703 CT Wageningen, The Netherlands.
SOURCE: Biochemical journal, (2002 Dec 1) 368 (Pt 2) 461-70. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20021121
Last Updated on STN: 20030118
Entered Medline: 20030117

AB The structural compatibility of two hyperthermostable family 1 glycoside hydrolases, **Pyrococcus** furiosus CelB and Sulfolobus solfataricus LacS, as well as their kinetic potential were studied by construction of a library of 2048 hybrid **beta-glycosidases** using DNA family shuffling. The hybrids were tested for their thermostability, ability to hydrolyse lactose and sensitivity towards inhibition by glucose. Three screening rounds at 70 degrees C led to the isolation of three high-performance hybrid enzymes (hybrid 11, 18 and 20) that had 1.5-3.5-fold and 3.5-8.6-fold increased lactose hydrolysis rates compared with parental CelB and LacS respectively. The three variants were the result of a single crossover event, which gave rise to hybrids with a LacS N-terminus and a main CelB sequence. Constructed three-dimensional models of the hybrid enzymes revealed that the catalytic (betaalpha)(8)-barrel was composed of both LacS and CelB elements. In addition, an extra intersubunit hydrogen bond in hybrids 18 and 20 might explain their superior stability over hybrid 11. This study demonstrates that extremely thermostable enzymes with limited homology and different mechanisms of stabilization can be efficiently shuffled to form stable hybrids with improved catalytic features.

L14 ANSWER 7 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:105928 SCISEARCH
THE GENUINE ARTICLE: 516EC
TITLE: Development of an ultrahigh-temperature process for the enzymatic hydrolysis of lactose. III. utilization of two thermostable **beta-glycosidases** in a continuous ultrafiltration membrane reactor and galacto-oligosaccharide formation under steady-state conditions
AUTHOR: Petzelbauer I; Splechtna B; Nidetzky B (Reprint)
CORPORATE SOURCE: Agr Univ Vienna, Inst Food Technol, Div Biochem Engn, Muthgasse 18, A-1190 Vienna, Austria (Reprint); Agr Univ Vienna, Inst Food Technol, Div Biochem Engn, A-1190 Vienna, Austria

COUNTRY OF AUTHOR: Austria
SOURCE: BIOTECHNOLOGY AND BIOENGINEERING, (15 FEB 2002) Vol. 77, No. 4, pp. 394-404.
Publisher: JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA.
ISSN: 0006-3592.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 34
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Hydrolysis of lactose by hyperthermophilic **beta-glycosidases** from the archaea Sulfolobus solfataricus (SsbetaGly) and **Pyrococcus** furiosus (CelB) was carried out at 70degreesC in a continuous stirred-tank reactor (CSTR) coupled to a 10-kDa cross-flow ultrafiltration module to recycle the enzyme. Recirculation rates of greater than or equal to1 min(-1), reaction of proteins with reducing sugars, and enzyme adsorption onto the membrane are major "operational" factors of enzyme inactivation in the CSTR. They cause the half-life times of SsbetaGly and CelB to be reduced two- and eight-fold, respectively, the average value for both enzymes now being similar to5 to 7 days. Using lactose at initial concentrations of 45 and 170 g/L, the CSTR was operated at a constant conversion level of similar to80% for more than 2 weeks without the occurrence of microbial contamination. The productivities for the SsbetaGly-catalyzed conversion of lactose were determined at different dilution rates and initial substrate concentrations, and exceed by a factor of greater than or equal to2 those observed with CelB under otherwise identical conditions. This difference reflects the approximately eight-fold stronger product inhibition of CelB by D-glucose. While the maximum total galacto-oligosaccharide production (90-100 mM) at 170 g/L lactose in the CSTR was not different from that in the batch reactor (CelB) or was greater by similar to25% (SsbetaGly), continuous and batchwise reactions with both enzymes differed markedly with regard to relative proportions of the individual saccharide components present at 80% substrate conversion. The CSTR yielded an up to four-fold greater ratio of disaccharides to trisaccharides concomitant with a 5- to 30-fold larger relative proportion of beta-D-Galp-(1-->3)-D-Glc in the product mixture. The results show that apart from continuous hydrolysis of lactose at 70degreesC, a CSTR charged with SsbetaGly or CelB and operated at steady-state conditions could be a useful reaction system for the production of galacto-oligosaccharides in which composition is narrower and more easily programmable, in terms of the individual components contained, as compared to the batchwise reaction. (C) 2002 John Wiley & Sons. Biotechnol Sioeng 77: 394-404, 2002; DOI 10.1002/bit.10106.

L14 ANSWER 8 OF 26 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-11227 BIOTECHDS
TITLE: Enzymatic cleavage of lactose using beta-galactosidase **beta-glycosidase** or beta-glucosidase for production of milk products with reduced lactose content; Sulfolobus solfataricus or **Pyrococcus** furiosus enzyme **expression** in Escherichia coli, Saccharomyces cerevisiae, Pichia stipitis or Lactococcus lactis
PATENT ASSIGNEE: Lactoprot
LOCATION: Austria.
PATENT INFO: AT 200000239 15 Apr 2001
APPLICATION INFO: AT 2000-239 17 Feb 2000
PRIORITY INFO: AT 1999-370 4 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-316525 [34]

AB Enzymatic cleavage of lactose by membrane diffusion reaction, carried out at 50-85 deg using a thermostable enzyme is claimed. Also claimed are: a similar process using a mesophilic enzyme at not over 50 deg, in which

the permeate is treated by sterile filtration and/or UV radiation; and production of milk products with reduce lactose content using the novel methods. In an example, skim milk was heated to 65 deg then passed through a hollow-fiber module along with a solution of **beta-glycosidase** and provided 80% hydrolysis of lactose. Others enzymes beta-galactosidase (EC-3.2.1.23) or beta-glucosidase (EC-3.2.1.21) derived from Sulfolobus solfataricus or **Pyrococcus** furiosus **expressed** in Escherichia coli BL21 DE3, Saccharomyces cerevisiae, Pichia stipitis or Lactococcus lactis were used. The above can be used to treat lactose solutions or milk, whey or their derivatives for human or animal use. (16pp)

L14 ANSWER 9 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:18108 SCISEARCH
THE GENUINE ARTICLE: 506BG
TITLE: Tailoring the substrate specificity of the **beta-glycosidase** from the thermophilic archaeon Sulfolobus solfataricus
AUTHOR: Corbett K; Fordham-Skelton A P; Gatehouse J A; Davis B G (Reprint)
CORPORATE SOURCE: Univ Durham, Dept Chem, South Rd, Durham DH1 3LE, England (Reprint); Univ Durham, Dept Chem, Durham DH1 3LE, England; Univ Durham, Dept Biol Sci, Durham DH1 3LE, England; Univ Durham, Res Ctr Biol Chem, Durham DH1 3LE, England; Univ Oxford, Dyson Perrins Lab, Oxford OX1 3QY, England; SERC, Daresbury Lab, CLRC, Warrington WA4 4AD, Cheshire, England
COUNTRY OF AUTHOR: England
SOURCE: FEBS LETTERS, (14 DEC 2001) Vol. 509, No. 3, pp. 355-360. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0014-5793.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 40

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The substrate specificity of the thermophilic **beta-glycosidase** (lacS) from the archaeon Sulfolobus solfataricus (SS betaG), a member of the glycohydrolase family 1, has been analysed at a molecular level using predictions from known protein sequences and structures and through site-directed mutagenesis. Three critical residues were identified and mutated to create catalysts with altered and broadened specificities for use in glycoside synthesis. The wild-type (WT) and mutated sequences were **expressed** as **recombinant** fusion proteins in Escherichia coli, with an added His(6)-tag to allow one-step chromatographic purification. Consistent with side-chain orientation towards OH-6, the single Met439 --> Cys mutation enhances D-xylosidase specificity 4.7-fold and decreases D-fucosidase activity 2-fold without greatly altering its activity towards other D-glycoside substrates. Glu432 --> Cys and Trp433 --> Cys mutations directed towards OH-4 and -3, respectively, more dramatically impair glucose (Glc), galactose (Gal), fucose specificity than for other glycosides, resulting in two glycosidases with greatly broadened substrate specificities. These include the first examples of stereospecificity tailoring in glycosidases (e.g. WT --> W433C, k(cat)/K-m (Gal):k(cat)/K-M (mannose (Man))=29.4:1 -->1.2:1). The robustness and high utility of these broad specificity SS betaG mutants in parallel synthesis were demonstrated by the formation of libraries of P-glycosides of Glc, Gal, xylose, Man in one-pot preparations at 50 degreesC in the presence of organic solvents, that could not be performed by SS betaG-WT. (C) 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V.

L14 ANSWER 10 OF 26 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-11839 BIOTECHDS

TITLE: Galactosyl transfer catalyzed by thermostable **beta-glycosidases** from Sulfolobus solfataticus and **Pyrococcus** furiosus: kinetics studies of the reactions of galactosyated enzyme intermediates with a range of nucleophiles;
plasmid-mediated gene transfer and **expression** in Escherichia coli and mathematical model
AUTHOR: Petzelbauer I; Splechtna B; *Nidetzky B
CORPORATE SOURCE: Univ.Vienna-Agr.Inst.Food-Technol.
LOCATION: Division of Biochemical Engineering, Institute of Food Technology, University of Agricultural Sciences (BOKU), Muthgasse 18, A-1190 Vienna, Austria.
Email: nide@edv2.boku.ac.at
SOURCE: J.Biochem.; (2001) 130, 3, 341-49
CODEN: JOBIAO
ISSN: 0021-924X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Galactosyl group transfer from an enzyme to neutral primary alcohols and azide was studied during reactions of thermostable **recombinant beta-glycosidases** from Sulfolobus solfataticus (Ss-beta-Gly) and **Pyrococcus** furiosus (CelB) with 2-nitrophenyl beta-D-galactopyranoside or lactose. The mathematical model was proposed of the reaction of galactosylated enzyme intermediates with a range of nucleophiles. Enzymes were produced by **expressing** plasmid-encoded structural genes into Escherichia coli and purifying the enzymes by the thermoprecipitation at 80 deg for 30 min, followed by anion exchange chromatography. The specific hydrolase activities of the enzymes were determined to be 600 U/mg (Ss-beta-Gly) and 2,000 U/mg (CelB). Results showed revealed that the nucleophile binding sites of both enzymes could be composed of several, but at least 2 subsites proximal to the catalytic site, sugar-binding sub-site-1. (38 ref)

L14 ANSWER 11 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2001:724811 SCISEARCH
THE GENUINE ARTICLE: 469LY
TITLE: Galactosyl transfer catalyzed by thermostable **beta -glycosidases** from Sulfolobus solfataricus and **Pyrococcus** furiosus: Kinetic studies of the reactions of galactosylated enzyme intermediates with a range of nucleophiles
AUTHOR: Petzelbauer I; Splechtna B; Nidetzky B (Reprint)
CORPORATE SOURCE: Univ Agr Sci, BOKU, Inst Food Technol, Div Biochem Engn, Muthgasse 18, A-1190 Vienna, Austria (Reprint); Univ Agr Sci, BOKU, Inst Food Technol, Div Biochem Engn, A-1190 Vienna, Austria
COUNTRY OF AUTHOR: Austria
SOURCE: JOURNAL OF BIOCHEMISTRY, (SEP 2001) Vol. 130, No. 3, pp. 341-349.
Publisher: JAPANESE BIOCHEMICAL SOC, ISHIKAWA BLDG-3F, 25-16 HONGO-5-CHOME, BUNKYO-KU, TOKYO, 113, JAPAN.
ISSN: 0021-924X.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 38

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The transfer of a galactosyl group from an enzyme to a number of neutral primary alcohols, phenol and azide has been studied during the reactions at 80 degreesC of thermostable **beta -glycosidases** from Sulfolobus solfataricus (Ss beta Gly) and **Pyrococcus** furiosus (CelB) with 2-nitrophenyl beta -D-galactopyranoside or lactose (4-O-beta -D-galactopyranosyl D-glucopyranose) as substrates. The rate constant ratios, k(Nu)/k(water), for partitioning of the galactosylated enzyme intermediates between

reaction with nucleophiles (k(Nu), M-1 s(-1)) and water (k(water), s(-1)) have been determined from the difference in the initial velocities of the formation of 2-nitrophenol or D-glucose, and D-galactose. The results show that hydrophobic bonding interactions contribute approximate to8 kJ mol(-1) to the stabilization of the transition state for the reaction of galactosylated enzyme intermediates of Ss beta Gly and CelB with 1-butanol, compared to the transition state for the enzymatic reaction with methanol. The leaving group/nucleophile binding sites of Ss beta Gly and CelB appear about 0.8 times as hydrophobic as n-octanol. Values of k(Nu)/k(water) for reactions of galactosylated Ss beta Gly with ethanol and substituted derivatives of ethanol show no clear dependence on the pK(a) of the primary hydroxy group of these nucleophiles in the pK(a) range 12.4-16.0. The binding of phenol with the galactosylated enzyme intermediates of Ss beta Gly and CelB occurs in a form that is mainly nonproductive pertaining to beta -galactoside synthesis. Neither enzyme catalyzes galactosyl transfer to azide ion. A model is proposed for the interaction of neutral nucleophiles at an extended acceptor site of the galactosylated en-zymes.

L14 ANSWER 12 OF 26 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:125831 HCAPLUS
DOCUMENT NUMBER: 134:363126
TITLE: Characterization of β-glycosylhydrolases from **Pyrococcus** furiosus
AUTHOR(S): Kaper, Thijs; Verhees, Corne H.; Lebbink, Joyce H. G.; Van Lieshout, Johan F. T.; Kluskens, Leon D.; Ward, Don E.; Kengen, Serve W. M.; Beerthuyzen, Marke M.; De Vos, Willem M.; Van der Oost, John
CORPORATE SOURCE: USA
SOURCE: Methods in Enzymology (2001), 330(Hyperthermophilic Enzymes, Part A), 329-346
CODEN: MENZAU; ISSN: 0076-6879
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Topics discussed include gene **cloning**; gene **expression** ; enzyme overprodn.; alternative production systems; protein purification; activity assay; and thermal inactivation. (c) 2001 Academic Press.
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 13 OF 26 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:627928 HCAPLUS
DOCUMENT NUMBER: 133:192395
TITLE: Method for enzymatically cleaving lactose, in particular, by using membrane diffusion reactors
INVENTOR(S): Novalic, Senad; Kulbe, Klaus Dieter
PATENT ASSIGNEE(S): Lactoprot Alpenlandische Milchindustrie und Handels-A.-G., Austria
SOURCE: PCT Int. Appl., 16 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000051441	A2	20000908	WO 2000-AT37	20000215
WO 2000051441	A3	20010301		

W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,

LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,
RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG,
US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE,
DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AT 9900370	A	20000215	AT 1999-370	19990304
EP 1158860	A2	20011205	EP 2000-904675	20000215
EP 1158860	B1	20020619		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO

AT 219331	E	20020715	AT 2000-904675	20000215
ES 2176166	T3	20021201	ES 2000-904675	20000215
AT 408447	B	20011126	AT 2000-239	20000217

PRIORITY APPLN. INFO.: AT 1999-370 A 19990304
WO 2000-AT37 W 20000215

AB The invention relates to a method for enzymically cleaving lactose, in particular, for enzymically cleaving lactose using membrane diffusion reactors. To this end, a thermostable enzyme or mixture of thermostable enzymes is used as an enzyme which cleaves lactose, and the method is carried out at a temperature ranging 60-80°C.

L14 ANSWER 14 OF 26 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:121661 HCAPLUS
DOCUMENT NUMBER: 132:177431
TITLE: Preparation of a heat-resistant .**beta**.-**glycosidase** of **Pyrococcus** horikoshii and **cloning** of its encoding gene
INVENTOR(S): Matsui, Ikuo; Ishikawa, Kazuhiko; Ishida, Hiroyasu; Kosugi, Keiji
PATENT ASSIGNEE(S): Agency of Industrial Sciences and Technology, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2000050870	A2	20000222	JP 1998-222866	19980806
JP 2995292	B2	19991227		
US 2002102635	A1	20020801	US 1999-369735	19990806

PRIORITY APPLN. INFO.: JP 1998-222866 A 19980806

AB The gene encoding a novel heat-resistant .**beta**.-**glycosidase** is isolated from **Pyrococcus** horikoshii strain JCM9974. The .**beta**.-**glycosidase** prepared from transgenic Escherichia coli exhibits a temperature optimum >100° and a pH optimum 6.0.

L14 ANSWER 15 OF 26 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2000495120 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10931188
TITLE: Transgalactosylation by thermostable **beta**-**glycosidases** from **Pyrococcus** furiosus and Sulfolobus solfataricus. Binding interactions of nucleophiles with the galactosylated enzyme intermediate make major contributions to the formation of new beta-glycosides during lactose conversion.
AUTHOR: Petzelbauer I; Reiter A; Splechtna B; Kosma P; Nidetzky B
CORPORATE SOURCE: Division of Biochemical Engineering, Institute of Food Technology and Institute of Chemistry, Universitat fur Bodenkultur Wien, Vienna, Austria.
SOURCE: European journal of biochemistry / FEBS, (2000 Aug) 267

(16) 5055-66.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20001027
Last Updated on STN: 20001027
Entered Medline: 20001018

AB The hyperthermostable **beta-glycosidases** from the Archaea Sulfolobus solfataricus (SsbetaGly) and **Pyrococcus** furiosus (CelB) hydrolyse beta-glycosides of D-glucose or D-galactose with relaxed specificities pertaining to the nature of the leaving group and the glycosidic linkage. To determine how specificity is manifested under conditions of kinetically controlled transgalactosylation, the major transfer products formed during the hydrolysis of lactose by these enzymes have been identified, and their appearance and degradation have been determined in dependence of the degree of substrate conversion. CelB and SsbetaGly show a marked preference for making new beta(1-->3) and beta(1-->6) glycosidic bonds by intermolecular as well as intramolecular transfer reactions. The intramolecular galactosyl transfer of CelB, relative to glycosidic-bond cleavage and release of glucose, is about 2.2 times that of SsbetaGly and yields beta-D-Galp-(1-->6)-D-Glc and beta-D-Galp-(1-->3)-D-Glc in a molar ratio of approximately 1 : 2. The partitioning of galactosylated SsbetaGly between reaction with sugars [kNu (M-1. s-1)] and reaction with water [kwater (s-1)] is about twice that of CelB. It gives a mixture of linear beta-D-glycosides, chiefly trisaccharides at early reaction times, in which the prevailing new glycosidic bonds are beta(1-->6) and beta(1-->3) for the reactions catalysed by SsbetaGly and CelB, respectively. The accumulation of beta-D-Galp-(1-->6)-D-Glc at the end of lactose hydrolysis reflects a 3-10-fold specificity of both enzymes for the hydrolysis of beta(1-->3) over beta(1-->6) linked glucosides. Galactosyl transfer from SsbetaGly or CelB to D-glucose occurs with partitioning ratios, kNu/kwater, which are seven and > 170 times those for the reactions of the galactosylated enzymes with 1-propanol and 2-propanol, respectively. Therefore, the binding interactions with nucleophiles contribute chiefly to formation of new beta-glycosides during lactose conversion. Likewise, noncovalent interactions with the glucose leaving group govern the catalytic efficiencies for the hydrolysis of lactose by both enzymes. They are almost fully **expressed** in the rate-limiting first-order rate constant for the galactosyl transfer from the substrate to the enzyme and lead to a positive deviation by approximately 2.5 log10 units from structure-reactivity correlations based on the pKa of the leaving group.

L14 ANSWER 16 OF 26 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:288276 BIOSIS
DOCUMENT NUMBER: PREV200000288276
TITLE: Comparative structural analysis and substrate specificity engineering of the hyperthermostable beta-glucosidase CelB from **Pyrococcus** furiosus.
AUTHOR(S): Kaper, Thijs [Reprint author]; Lebbink, Joyce H.G.; Pouwels, Jeroen; Kopp, Juergen; Schulz, Georg E.; van der Oost, John; de Vos, Willem M.
CORPORATE SOURCE: Laboratory of Microbiology, Department of Biomolecular Sciences, Wageningen University, Hesselink van Suchtelenweg 4, NL-6703 CT, Wageningen, Netherlands
SOURCE: Biochemistry, (May 2, 2000) Vol. 39, No. 17, pp. 4963-4970. print.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Jul 2000

Last Updated on STN: 7 Jan 2002

AB The substrate specificity of the beta-glucosidase (CelB) from the hyperthermophilic archaeon **Pyrococcus** furiosus, a family 1 glycosyl hydrolase, has been studied at a molecular level. Following crystallization and X-ray diffraction of this enzyme, a 3.3 ANG resolution structural model has been obtained by molecular replacement. CelB shows a homo-tetramer configuration, with subunits having a typical (betaalpha)8-barrel fold. Its active site has been compared to the one of the previously determined 6-phospho-**beta-glycosidase** (LacG) from the mesophilic bacterium Lactococcus lactis. The overall design of the substrate binding pocket is very well conserved, with the exception of three residues that have been identified as a phosphate binding site in LacG. To verify the structural model and alter its substrate specificity, these three residues have been introduced at the corresponding positions in CelB (E417S, M424K, F426Y) in different combinations: single, double, and triple mutants. Characterization of the purified mutant CelB enzyme revealed that F426Y resulted in an increased affinity for galactosides, whereas M424K gave rise to a shifted pH optimum (from 5.0 to 6.0). Analysis of E417S revealed a 5-fold and a 3-fold increase of the efficiency of hydrolyzing o-nitrophenol-beta-D-galactopyranoside-6-phosphate, in the single and triple mutants, respectively. In contrast, their activity on nonphosphorylated sugars was largely reduced (30-300-fold). The residue at position E417 in CelB seems to be the determining factor for the difference in substrate specificity between the two types of family 1 glycosidases.

L14 ANSWER 17 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2001:177935 SCISEARCH
THE GENUINE ARTICLE: 403MB
TITLE: Kinetic study of a thermostable **beta-glycosidase** of Thermus thermophilus. Effects of temperature and glucose on hydrolysis and transglycosylation reactions
AUTHOR: Fourage L; Dion M; Colas B (Reprint)
CORPORATE SOURCE: Fac Sci & Tech, CNRS, FRE 2230, Unite Rech Biocatalyse, 2 Rue Houssiniere, BP 92208, F-44322 Nantes 3, France (Reprint); Fac Sci & Tech, CNRS, FRE 2230, Unite Rech Biocatalyse, F-44322 Nantes 3, France
COUNTRY OF AUTHOR: France
SOURCE: GLYCOCONJUGATE JOURNAL, (JUN 2000) Vol. 17, No. 6, pp. 377-383.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
ISSN: 0282-0080.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 37

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A **beta -glycosidase** of a thermophile, Thermus thermophilus, belonging to the glycoside hydrolase family 1, was **cloned** and overexpressed in Escherichia coli. The purified enzyme (Tt beta gly) has a broad substrate specificity towards beta -D-glucoside, beta -D-galactoside and beta -D-fucoside derivatives. The thermostability of Tt beta gly was exploited to study its kinetic properties within the range 25-80 degreesC. Whatever the temperature, except around 60 degreesC, the enzyme displayed non-Michaelian kinetic behavior. Tt beta gly was inhibited by high concentrations of substrate below 60 degreesC and was activated by high concentrations of substrate above 60 degreesC. The apparent kinetic parameters (k(cat) and K-m) were calculated at different temperatures. Both k(c)at and K-m increased with an increase in temperature, but up to 75 degreesC the values of k(cat) increased much more rapidly than the values of K-m. The observed kinetics might be due to a combination of factors including inhibition by excess substrate and stimulation due to transglycosylation reactions. Our results show that the

substrate could act not only as a glycosyl donor but also as a glycosyl acceptor. In addition, when the glucose was added to reaction mixtures, inhibition or activation was observed depending on both substrate concentration and temperature. A reaction model is proposed to explain the kinetic behavior of Tt beta gly. The scheme integrates the inhibition observed at high concentrations of substrate and the activation due to transglycosylation reactions implicating the existence of a transfer subsite.

L14 ANSWER 18 OF 26 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2000141228 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10675537
TITLE: Novel substrate specificity of a membrane-bound **beta-glycosidase** from the hyperthermophilic archaeon **Pyrococcus** horikoshii.
AUTHOR: Matsui I; Sakai Y; Matsui E; Kikuchi H; Kawarabayasi Y; Honda K
CORPORATE SOURCE: National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki, Japan.. ikmatsui@nibh.go.jp
SOURCE: FEBS letters, (2000 Feb 11) 467 (2-3) 195-200. Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000413
Last Updated on STN: 20000413
Entered Medline: 20000331
AB A **beta-glycosidase** gene homolog of **Pyrococcus** horikoshii (BGPh) was successfully **expressed** in Escherichia coli. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85 degrees C for 15 min. The optimum pH was 6.0 and the optimum temperature was over 100 degrees C, respectively. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90 degrees C (pH 6.0) was 15 h. BGPh has a novel substrate specificity with k(cat)/K(m) values high enough for hydrolysis of beta-D-Glcp derivatives with long alkyl chain at the reducing end and low enough for the hydrolysis of beta-linked glucose dimer more hydrophilic than aryl- or alkyl-beta-D-Glcp.

L14 ANSWER 19 OF 26 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2000-09594 BIOTECHDS
TITLE: Development of an ultra-high-temperature process for the enzymatic hydrolysis of lactose: II. Oligosaccharide formation by two thermostable **beta-glycosidases**;
Sulfolobus solfataricus and **Pyrococcus** furiosus **recombinant beta-glycosidase** production via vector-mediated gene transfer and **expression** in Escherichia coli
AUTHOR: Petzelbauer I; Zeleny R; Reiter A; Kulbe K D; *Nidetzky B
CORPORATE SOURCE: Univ.Vienna-Agr.Inst.Food-Technol.; Inst.Anim.Biotechnol.Tulln; Univ.Vienna-Agr.Inst.Chem.
LOCATION: Division of Biochemical Engineering, Institute of Food Technology, Universitat fur Bodenkultur Vienna (BOKU), Muthgasse 18, A-1190 Vienna, Austria.
Email: nide@edv2.boku.ac.at
SOURCE: Biotechnol.Bioeng.; (2000) 69, 2, 140-49
CODEN: BIBIAU
ISSN: 0006-3592
DOCUMENT TYPE: Journal
LANGUAGE: English
AB During the conversion of lactose at 70 deg, catalyzed by **beta-**

glycosidases from Sulfolobus solfataricus (Ss-beta-Gly) and **Pyrococcus** furiosus (DSM 3658) (CelB), the transfer of galactosyl to acceptors other than water competes efficiently with complete hydrolysis of the substrate. Ss-beta-Gly and CelB were produced recombinantly in Escherichia coli cells. The conversion of lactose led to the transient formation of a range of new products, mainly disaccharides and trisaccharides and displayed a marked dependence on the initial substrate concentration as well as on the degree of lactose conversion. The oligosaccharides produced were analyzed quantitatively using capillary electrophoresis and HPLC. When an initial lactose concentration of 270 g/l was used, a maximum concentration of 86 g/l were accumulated at 80% lactose conversion and with both enzymes the molar ratio of trisaccharides to disaccharides was maximal at an early stage of reaction and it decreased directly proportional to an increase in substrate conversion. Of the 2 enzymes CelB produced around 6% more hydrolysis byproducts, but the spectrum of products produced by Ss-beta-Glys had more trisacchrides. (52 ref)

L14 ANSWER 20 OF 26 MEDLINE on STN DUPLICATE 8

ACCESSION NUMBER: 1999326267 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10397869
TITLE: Development of an ultra-high-temperature process for the enzymatic hydrolysis of lactose. I. The properties of two thermostable **beta-glycosidases**.
COMMENT: Erratum in: Biotechnol Bioeng 1999 Dec 20;65(6):following 676
AUTHOR: Petzelbauer I; Nidetzky B; Haltrich D; Kulbe K D
CORPORATE SOURCE: Division of Biochemical Engineering, Institute of Food Technology, Universitat fur Bodenkultur Wien (BOKU), Muthgasse 18, A-1190 Wien, Austria.
SOURCE: Biotechnology and bioengineering, (1999 Aug 5) 64 (3) 322-32.
Journal code: 7502021. ISSN: 0006-3592.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990925
Last Updated on STN: 20000327
Entered Medline: 19990913

AB **Recombinant beta-glycosidases** from hyperthermophilic Sulfolobus solfataricus (SsbetaGly) and **Pyrococcus** furiosus (CelB) have been characterized with regard to their potential use in lactose hydrolysis at about 70 degrees C or greater. Compared with SsbetaGly, CelB is approximately 15 times more stable against irreversible denaturation by heat, its operational half-life time at 80 degrees C and pH 5.5 being 22 days. The stability of CelB but not that of SsbetaGly is decreased 4-fold in the presence of 200 mM lactose at 80 degrees C. CelB displays a broader pH/activity profile than SsbetaGly, retaining at least 60% enzyme activity between pH 4 and 7. Both enzymes have a similar activation energy for lactose hydrolysis of approximately 75 kJ/mol (pH 5.5), and this is constant between 30 and 95 degrees C. D-Galactose is a weak competitive inhibitor against the release of D-glucose from lactose (Ki approximately 0.3 M), and at 80 degrees C the ratio of Ki, D-galactose to Km,lactose is 2.5 and 4.0 for CelB and SsbetaGly, respectively. SsbetaGly is activated up to 2-fold in the presence of D-glucose with respect to the maximum rate of glycosidic bond cleavage, measured with o-nitrophenyl beta-D-galactoside as the substrate. By contrast, CelB is competitively inhibited by D-glucose and has a Ki of 76 mM. The transfer of the galactosyl group from lactose to acceptors such as lactose or D-glucose rather than water is significant for both enzymes and depends on the initial lactose concentration as well as the time-dependent substrate/product ratio during batchwise lactose

conversion. It is approximately 1.8 times higher for SsbetaGly, compared with CelB. Overall, CelB and SsbetaGly share their catalytic properties with much less thermostable **beta-glycosidases** and thus seem very suitable for lactose hydrolysis at >/=70 degrees C. Copyright 1999 John Wiley & Sons, Inc.

L14 ANSWER 21 OF 26 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 9

ACCESSION NUMBER: 1999327626 EMBASE
TITLE: Gene analysis and enzymatic properties of thermostable . **beta.-glycosidase** from **Pyrococcus** kodakaraensis KOD1.
AUTHOR: Ezaki S.; Miyaoku K.; Nishi K.-I.; Tanaka T.; Fujiwara S.; Takagi M.; Atomi H.; Imanaka T.
CORPORATE SOURCE: T. Imanaka, Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
SOURCE: Journal of Bioscience and Bioengineering, (1999) 88/2 (130-135).
Refs: 22
ISSN: 1389-1723 CODEN: JBBIF6
COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A .**beta.-glycosidase** with broad substrate specificity was identified from a hyperthermophilic archaeon, **Pyrococcus** kodakaraensis KOD1. The gene encoding .**beta.-glycosidase** (Pk-gly) consists of 1449 nucleotides corresponding to a polypeptide of 483 amino acids. The protein showed similarity with other .**beta** .- **glycosidases** from family-1 glycosyl hydrolases, in particular, it showed high identity to β-mannosidase from P. furiosus (55.7%), .**beta.-glycosidase** from Sulfolobus solfataricus (42.7%) and β-glucosidase from P. furiosus (41.9%). The **cloned** gene was **expressed** in Escherichia coli and the **recombinant** protein was purified. The .**beta.-glycosidase** showed optimal activity at pH 6,5 and at an extremely high temperature of 100°C, and had a half-life of 18 h at 90°C. The .**beta.-glycosidase** hydrolyzed various pNp-β-glycopyranosides, with k(cat)/K(m) values in the order of pNp-β-glucopyranoside dot left right equal to pNp-β-mannopyranoside>pNp-β-galactopyranoside>pNp-β- xylopyranoside. pNp-β-mannopyranoside was the substrate exhibiting the lowest K(m) value [0.254 mM] with a k(cat)/K(m) ratio comparable to that of pNp-β-glucopyranoside. This substrate specificity was distinct from previously reported .**beta.-glycosidases**. We observed that the region in Pk-Gly corresponding to the fifth α-helix and β-strand region of . **beta.-glycosidase** from S. solfataricus, which constitutes a large portion of the channel for substrate incorporation, displayed a chimeric structure, with the N-terminal region similar to .**beta.-glycosidases** and the C-terminal region similar to β- mannosidases. An exo-type hydrolytic activity and transglycosylation activity were also observed towards cellooligomers.

L14 ANSWER 22 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1999:496230 SCISEARCH
THE GENUINE ARTICLE: 208XA
TITLE: **Cloning** and **expression** of a **beta-glycosidase** gene from Thermus thermophilus. Sequence and biochemical characterization of the encoded enzyme

AUTHOR: Dion M; Fourage L; Hallet J N; Colas B (Reprint)
CORPORATE SOURCE: UNIV NANTES, FAC SCI & TECH, UNITE RECH BIOCATALYSE, 2 RUE HOUSSINIERE, BP 92208, F-44322 NANTES 3, FRANCE (Reprint); UNIV NANTES, FAC SCI & TECH, UNITE RECH BIOCATALYSE, F-44322 NANTES 3, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: GLYCOCONJUGATE JOURNAL, (JAN 1999) Vol. 16, No. 1, pp. 27-37.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
ISSN: 0282-0080.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 67

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A 3.2 kilobase pair DNA fragment from Thermus thermophilus HB27 coding for a beta-galactosidase activity was **cloned** and sequenced. A gene and a truncated open reading frame orf1 encoding respectively a **beta-glycosidase** (tt beta-gly) and probably a sugar permease were located directly adjacent to each other. The deduced aminoacid sequence of the enzyme Tt beta-gly showed strong identity with those of **beta-glycosidases** belonging to the glycosyl hydrolase family 1. The enzyme was overexpressed in Escherichia coli and was purified by a two-step purification procedure. The **recombinant** enzyme is monomeric with a molecular mass of 49-kDa. It catalyzes the hydrolysis of beta-D-galactoside, beta-D-glucoside and beta-D-fucoside derivatives. However, the kcat/Km ratio is much higher for p-nitrophenyl-beta-D-glucoside and p-nitrophenyl-beta-D-fucoside than for p-nitrophenyl-beta-D-galactoside. The specificity towards linkage positions of the disaccharides tested decreased in the following order: beta 1-3 (100%). beta 1-2 (71%). beta 1-4 (40%). beta 1-6 (10%). Tt beta-gly is a thermostable enzyme displaying an optimum temperature of 88 degrees C and a half life of 10 min at 90 degrees C. It performs transglycosylation reactions at high temperature with a yield exceeding 63% for transfucosylation reactions. On the basis of this work, the enzyme appears to be an attractive tool in the synthesis of fucosyl adducts and fucosyl sugars.

L14 ANSWER 23 OF 26 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 1998058904 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9395451
TITLE: Molecular and biochemical characterization of an endo-beta-1,3- glucanase of the hyperthermophilic archaeon **Pyrococcus** furiosus.
AUTHOR: Gueguen Y; Voorhorst W G; van der Oost J; de Vos W M
CORPORATE SOURCE: Bacterial Genetics Group, Department of Microbiology, Wageningen Agricultural University, Hesselink van Suchtelenweg 4, NL-6703 CT Wageningen, The Netherlands.
SOURCE: Journal of biological chemistry, (1997 Dec 12) 272 (50) 31258-64.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF013169
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980129
Last Updated on STN: 19980129
Entered Medline: 19980115

AB We report here the first molecular characterization of an endo-beta-1,3-glucanase from an archaeon. **Pyrococcus** furiosus is a hyperthermophilic archaeon that is capable of saccharolytic growth.

The isolated lamA gene encodes an extracellular enzyme that shares homology with both endo-beta-1,3- and endo-beta-1,3-1,4-glucanases of the glycosyl hydrolase family 16. After deletion of the N-terminal leader sequence, a lamA fragment encoding an active endo-beta-1,3-glucanase was overexpressed in Escherichia coli using the T7-**expression** system. The purified P. furiosus endoglucanase has highest hydrolytic activity on the beta-1,3-glucose polymer laminarin and has some hydrolytic activity on the beta-1,3-1,4 glucose polymers lichenan and barley beta-glucan. The enzyme is the most thermostable endo-beta-1,3-glucanase described up to now; it has optimal activity at 100-105 degrees C. In the predicted active site of glycosyl hydrolases of family 16 that show predominantly endo-beta-1,3-glucanase activity, an additional methionine residue is present. Deletion of this methionine did not change the substrate specificity of the endoglucanase, but it did cause a severe reduction in its catalytic activity, suggesting a structural role of this residue in constituting the active site. High performance liquid chromatography analysis showed in vitro hydrolysis of laminarin by the endo-beta-1,3-glucanase proceeds more efficiently in combination with an exo-**beta-glycosidase** from P. furiosus (CelB). This most probably reflects the physiological role of these enzymes: cooperation during growth of P. furiosus on beta-glucans.

L14 ANSWER 24 OF 26 MEDLINE on STN DUPLICATE 11

ACCESSION NUMBER: 96394494 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8798600
TITLE: Comparison of a beta-glucosidase and a beta-mannosidase from the hyperthermophilic archaeon **Pyrococcus** furiosus. Purification, characterization, gene **cloning**, and sequence analysis.
AUTHOR: Bauer M W; Bylina E J; Swanson R V; Kelly R M
CORPORATE SOURCE: Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, USA.
SOURCE: Journal of biological chemistry, (1996 Sep 27) 271 (39) 23749-55.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U60214
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19980206
Entered Medline: 19961118

AB Two distinct exo-acting, beta-specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus** furiosus: a beta-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213, 305-312), and a beta-mannosidase. The beta-mannosidase and beta-glucosidase genes were isolated from a genomic library by **expression** screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the beta-mannosidase and the beta-glucosidase, respectively. The beta-glucosidase gene was identical to that reported by Voorhorst et al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J., and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession number U37557U37557). The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the **beta-glycosidases** from Sulfolobus solfataricus, Thermotoga maritima, and Caldocellum saccharolyticum. Based on these sequence similarities, the beta-mannosidase and the beta-glucosidase can both be classified as family 1 glycosyl hydrolases. In addition, the beta-mannosidase and

beta-glucosidase from P. furiosus both contained the conserved active site residues found in all family 1 enzymes. The beta-mannosidase showed optimal activity at pH 7.4 and 105 degrees C. Although the enzyme had a half-life of greater than 60 h at 90 degrees C, it is much less thermostable than the beta-glucosidase, which had a reported half-life of 85 h at 100 degrees C. Km and Vmax values for the beta-mannosidase were determined to be 0.79 mM and 31.1 micromol para-nitrophenol released/min/mg with p-nitrophenyl-beta-D-mannopyranoside as substrate. The catalytic efficiency of the beta-mannosidase was significantly lower than that reported for the P. furiosus beta-glucosidase (5.3 versus 4, 500 s-1 mM-1 with p-nitrophenyl-beta-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the beta-glucosidase, the primary role of the beta-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L14 ANSWER 25 OF 26 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 96099293 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8522516
TITLE: Characterization of the celB gene coding for beta-glucosidase from the hyperthermophilic archaeon **Pyrococcus** furiosus and its **expression** and site-directed mutation in Escherichia coli.
AUTHOR: Voorhorst W G; Eggen R I; Luesink E J; de Vos W M
CORPORATE SOURCE: Department of Microbiology, Wageningen Agricultural University, The Netherlands.
SOURCE: Journal of bacteriology, (1995 Dec) 177 (24) 7105-11. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U37557; PIR-A27233; PIR-A28673; PIR-A29897; PIR-B37168; SWISSPROT-P14288; SWISSPROT-P22498; SWISSPROT-S03813
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960219
Last Updated on STN: 19980206
Entered Medline: 19960122

AB The celB gene encoding the cellobiose-hydrolyzing enzyme beta-glucosidase from the hyperthermophilic archaeon **Pyrococcus** furiosus has been identified, **cloned**, and sequenced. The transcription and translation gene was overexpressed in Escherichia coli, resulting in high-level (up to 20% of total protein) production of beta-glucosidase that could be purified by a two-step purification procedure. The beta-glucosidase produced by E. coli had kinetic and stability properties similar to those of the beta-glucosidase purified from P. furiosus. The deduced amino acid sequence of CelB showed high similarity with those of **beta-glycosidases** that belong to glycosyl hydrolase family 1, implicating a conserved structure. Replacement of the conserved glutamate 372 in the P. furiosus beta-glucosidase by an aspartate or a glutamine led to a high reduction in specific activity (200- or 1,000-fold, respectively), indicating that this residue is the active site nucleophile involved in catalysis above 100 degrees C.

L14 ANSWER 26 OF 26 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1994-13129 BIOTECHDS
TITLE: Extremophiles in biotechnology; production and application of e.g. thermophilic bacterium and its enzyme (conference paper)
AUTHOR: Rossi M; De Rosa M
CORPORATE SOURCE: CNR-Inst.Biochem.Protein-Enzymol.; Univ.Naples; Univ.Naples-Inst.Biochem.Macromol.
LOCATION: Istituto di Biochimica delle Proteine ed Enzimologie, CNR,

Via Marconi 10, 80125 Napoli, Italy.
SOURCE: Prog.Biotechnol.; (1994) 9, Pt.1, 255-62
CODEN: PBITE3
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The use of enzymes and the availability of extremophiles as a source of enzymes stable and active at high temperature and in adverse chemical environments are important in the potential exploitation of extremophiles and their enzymes in biotechnology. Thermophiles appear to be the most interesting and promising organisms for these purposes. A table was presented on the thermal stability of some thermophilic enzymes, e.g. proteases from Thermus sp. Rt41A, Thermococcus celer, Desulfurococcus sp. and **Pyrococcus** furiosus with half lives of 13.5 hr at 80 deg, 45 hr at 95 deg, 1.5 hr at 95 deg and 33 hr and 95 deg, respectively. A general methodology was set up for the production of archaebacterial enzymes, which involved **cloning** enzymatic activities (**beta-glycosidase**, DNA-polymerase (EC-2.7.7.7) and alcohol-dehydrogenase (EC-1.1.1.1)) from Sulfolobus solfataricus and **expression** in Escherichia coli, yeast or human cells. Several processes may use thermostable cellulases (EC-3.2.1.4), hemicellulases and amylases in the future e.g. production of liquid fuels from renewable biomass, food and fruit juice processing, paper manufacture, etc. (21 ref)

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=> e kosugi y/au
E1            2     KOSUGI W/AU
E2            2     KOSUGI WATARU/AU
E3          460 --> KOSUGI Y/AU
E4            1     KOSUGI Y */AU
E5            7     KOSUGI YAE/AU
E6           10     KOSUGI YASUHIKO/AU
E7            1     KOSUGI YASUHIRO/AU
E8            5     KOSUGI YASUHITO/AU
E9            1     KOSUGI YASUMASA/AU
E10           1     KOSUGI YASUNORI/AU
E11           1     KOSUGI YASUO/AU
E12           2     KOSUGI YASUSHI/AU

=> s e3
L15         460 "KOSUGI Y"/AU

=> e ishida h/au
E1            6     ISHIDA GORO/AU
E2            1     ISHIDA GOROU/AU
E3         6017 --> ISHIDA H/AU
E4            1     ISHIDA H */AU
E5            1     ISHIDA H HIDEKI/AU
E6            4     ISHIDA H I/AU
E7           13     ISHIDA H K/AU
E8            2     ISHIDA H S/AU
E9            1     ISHIDA H Y/AU
E10           1     ISHIDA HAITOSHI/AU
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E12           1     ISHIDA HAKASE/AU

=> s e3
L16        6017 "ISHIDA H"/AU

=> e ishikawa k/au
E1            1     ISHIKAWA JYUNKO/AU
E2            1     ISHIKAWA JYUZABURO/AU
E3         8442 --> ISHIKAWA K/AU
E4            5     ISHIKAWA K */AU
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E5           18      ISHIKAWA K B/AU
E6            1      ISHIKAWA K H/AU
E7          226      ISHIKAWA K I/AU
E8            1      ISHIKAWA K J/AU
E9            2      ISHIKAWA K L/AU
E10           1      ISHIKAWA K N/AU
E11           4      ISHIKAWA K S/AU
E12           4      ISHIKAWA K Y/AU

=> s e3
L17        8442 "ISHIKAWA K"/AU

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E1            1      MATSUI HOROYUKI/AU
E2            1      MATSUI HROSHI/AU
E3          637 --> MATSUI I/AU
E4            1      MATSUI I G/AU
E5            1      MATSUI I KOJI/AU
E6            1      MATSUI I M/AU
E7            1      MATSUI I MASANAO/AU
E8            1      MATSUI I S/AU
E9            3      MATSUI I S L/AU
E10           1      MATSUI ICHINOSUKE/AU
E11          91      MATSUI ICHIRO/AU
E12           1      MATSUI ICHIZO/AU

=> s e3
L18         637 "MATSUI I"/AU

=> d his

     (FILE 'HOME' ENTERED AT 10:21:50 ON 10 JUN 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:22:13 ON 10 JUN 2004
L1          1757 S BETA(W)GLYCOSIDASE?
L2         53153 S TETRAMER
L3            32 S L1 AND L2
L4             9 DUP REM L3 (23 DUPLICATES REMOVED)
L5          7080 S PYROCOCCUS
L6           156 S L1 AND L5
L7             8 S L2 AND L6
L8             3 DUP REM L7 (5 DUPLICATES REMOVED)
L9           741 S L5(A)HORIKOSHII
L10            6 S L1 AND L9
L11            2 DUP REM L10 (4 DUPLICATES REMOVED)
L12      6555393 S CLON? OR EXPRESS? OR RECOMBINANT
L13           75 S L6 AND L12
L14           26 DUP REM L13 (49 DUPLICATES REMOVED)
                 E KOSUGI Y/AU
L15          460 S E3
                 E ISHIDA H/AU
L16         6017 S E3
                 E ISHIKAWA K/AU
L17         8442 S E3
                 E MATSUI I/AU
L18          637 S E3

=> s l14 or l15 or l16 or l17 or l18
L19       15412 L14 OR L15 OR L16 OR L17 OR L18

=> s l1 and l19
L20          29 L1 AND L19
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=> dup rem l20
PROCESSING COMPLETED FOR L20
L21 26 DUP REM L20 (3 DUPLICATES REMOVED)

=> d 1-26 ibib

L21 ANSWER 1 OF 26 MEDLINE on STN
ACCESSION NUMBER: 2003361724 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12859194
TITLE: Activity of hyperthermophilic glycosynthases is significantly enhanced at acidic pH.
AUTHOR: Perugino Giuseppe; Trincone Antonio; Giordano Assunta; van der Oost John; Kaper Thijs; Rossi Mose; Moracci Marco
CORPORATE SOURCE: Institute of Protein Biochemistry-Consiglio Nazionale delle Ricerche, Via P. Castellino 111, 80131, Naples Italy.
SOURCE: Biochemistry, (2003 Jul 22) 42 (28) 8484-93.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200310
ENTRY DATE: Entered STN: 20030805
Last Updated on STN: 20031008
Entered Medline: 20031006

L21 ANSWER 2 OF 26 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:563403 BIOSIS
DOCUMENT NUMBER: PREV200200563403
TITLE: The temperature influences the ratio of glucosidase and galactosidase activities of **beta-glycosidases**.
AUTHOR(S): Hansson, Therese; Adlercreutz, Patrick [Reprint author]
CORPORATE SOURCE: Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, SE-221 00, P.O. Box 124, Lund, Sweden
patrick.adlercreutz@biotek.lu.se
SOURCE: Biotechnology Letters, (September, 2002) Vol. 24, No. 18, pp. 1465-1471. print.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Oct 2002
Last Updated on STN: 30 Oct 2002

L21 ANSWER 3 OF 26 MEDLINE on STN
ACCESSION NUMBER: 2002080570 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11807757
TITLE: Development of an ultrahigh-temperature process for the enzymatic hydrolysis of lactose. IV. Immobilization of two thermostable **beta-glycosidases** and optimization of a packed-bed reactor for lactose conversion.
AUTHOR: Petzelbauer Inge; Kuhn Bernhard; Splechtna Barbara; Kulbe Klaus D; Nidetzky Bernd
CORPORATE SOURCE: Division of Biochemical Engineering, Institute of Food Technology, Universitat fur Bodenkultur Wien (BOKU), Muthgasse 18, A-1190 Vienna, Austria.
SOURCE: Biotechnology and bioengineering, (2002 Mar 20) 77 (6) 619-31.
Journal code: 7502021. ISSN: 0006-3592.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020128
Last Updated on STN: 20020518
Entered Medline: 20020517

L21 ANSWER 4 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:119696 SCISEARCH
THE GENUINE ARTICLE: 517AH
TITLE: Regulation of endo-acting glycosyl hydrolases in the hyperthermophilic bacterium Thermotoga maritima grown on glucan- and mannan-based polysaccharides
AUTHOR: Chhabra S R; Shockley K R; Ward D E; Kelly R M (Reprint)
CORPORATE SOURCE: N Carolina State Univ, Dept Chem Engn, Stinson Dr, Box 7905, Raleigh, NC 27695 USA (Reprint); N Carolina State Univ, Dept Chem Engn, Raleigh, NC 27695 USA
COUNTRY OF AUTHOR: USA
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (FEB 2002) Vol. 68, No. 2, pp. 545-554.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0099-2240.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 56
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L21 ANSWER 5 OF 26 MEDLINE on STN
ACCESSION NUMBER: 2002277402 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12018273
TITLE: Hydrolysis of lactose by **beta-glycosidase** CelB from hyperthermophilic archaeon **Pyrococcus** furiosus: comparison of hollow-fiber membrane and packed-bed immobilized enzyme reactors for continuous processing of ultrahigh temperature-treated skim milk.
AUTHOR: Splechtna Barbara; Petzelbauer Inge; Kuhn Bernhard; Kulbe Klaus D; Nidetzky Bernd
CORPORATE SOURCE: Division of Biochemical Engineering, Institute of Food Technology, University of Agricultural Sciences, Vienna, Austria.
SOURCE: Applied biochemistry and biotechnology, (2002 Spring) 98-100 473-88.
Journal code: 8208561. ISSN: 0273-2289.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20020522
Last Updated on STN: 20021211
Entered Medline: 20021106

L21 ANSWER 6 OF 26 MEDLINE on STN
ACCESSION NUMBER: 2002680611 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12164784
TITLE: DNA family shuffling of hyperthermostable **beta-glycosidases**.
AUTHOR: Kaper Thijs; Brouns Stan J J; Geerling Ans C M; De Vos Willem M; Van der Oost John
CORPORATE SOURCE: Laboratory of Microbiology, Wageningen University, Hessenlink van Suchtelenweg 4, NL-6703 CT Wageningen, The Netherlands.
SOURCE: Biochemical journal, (2002 Dec 1) 368 (Pt 2) 461-70.
Journal code: 2984726R. ISSN: 0264-6021.

PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20021121
Last Updated on STN: 20030118
Entered Medline: 20030117

L21 ANSWER 7 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:105928 SCISEARCH
THE GENUINE ARTICLE: 516EC
TITLE: Development of an ultrahigh-temperature process for the enzymatic hydrolysis of lactose. III. utilization of two thermostable **beta-glycosidases** in a continuous ultrafiltration membrane reactor and galacto-oligosaccharide formation under steady-state conditions
AUTHOR: Petzelbauer I; Splechtna B; Nidetzky B (Reprint)
CORPORATE SOURCE: Agr Univ Vienna, Inst Food Technol, Div Biochem Engn, Muthgasse 18, A-1190 Vienna, Austria (Reprint); Agr Univ Vienna, Inst Food Technol, Div Biochem Engn, A-1190 Vienna, Austria
COUNTRY OF AUTHOR: Austria
SOURCE: BIOTECHNOLOGY AND BIOENGINEERING, (15 FEB 2002) Vol. 77, No. 4, pp. 394-404.
Publisher: JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA.
ISSN: 0006-3592.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 34
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L21 ANSWER 8 OF 26 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-11227 BIOTECHDS
TITLE: Enzymatic cleavage of lactose using beta-galactosidase **beta-glycosidase** or beta-glucosidase for production of milk products with reduced lactose content; Sulfolobus solfataricus or **Pyrococcus** furiosus enzyme **expression** in Escherichia coli, Saccharomyces cerevisiae, Pichia stipitis or Lactococcus lactis
PATENT ASSIGNEE: Lactoprot
LOCATION: Austria.
PATENT INFO: AT 200000239 15 Apr 2001
APPLICATION INFO: AT 2000-239 17 Feb 2000
PRIORITY INFO: AT 1999-370 4 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-316525 [34]

L21 ANSWER 9 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:18108 SCISEARCH
THE GENUINE ARTICLE: 506BG
TITLE: Tailoring the substrate specificity of the **beta-glycosidase** from the thermophilic archaeon Sulfolobus solfataricus
AUTHOR: Corbett K; Fordham-Skelton A P; Gatehouse J A; Davis B G (Reprint)
CORPORATE SOURCE: Univ Durham, Dept Chem, South Rd, Durham DH1 3LE, England (Reprint); Univ Durham, Dept Chem, Durham DH1 3LE, England; Univ Durham, Dept Biol Sci, Durham DH1 3LE, England; Univ Durham, Res Ctr Biol Chem, Durham DH1 3LE,

England; Univ Oxford, Dyson Perrins Lab, Oxford OX1 3QY, England; SERC, Daresbury Lab, CLRC, Warrington WA4 4AD, Cheshire, England
COUNTRY OF AUTHOR: England
SOURCE: FEBS LETTERS, (14 DEC 2001) Vol. 509, No. 3, pp. 355-360. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0014-5793.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 40
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L21 ANSWER 10 OF 26 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-11839 BIOTECHDS
TITLE: Galactosyl transfer catalyzed by thermostable **beta-glycosidases** from Sulfolobus solfataticus and **Pyrococcus** furiosus: kinetics studies of the reactions of galactosyated enzyme intermediates with a range of nucleophiles; plasmid-mediated gene transfer and **expression** in Escherichia coli and mathematical model
AUTHOR: Petzelbauer I; Splechtna B; *Nidetzky B
CORPORATE SOURCE: Univ.Vienna-Agr.Inst.Food-Technol.
LOCATION: Division of Biochemical Engineering, Institute of Food Technology, University of Agricultural Sciences (BOKU), Muthgasse 18, A-1190 Vienna, Austria. Email: nide@edv2.boku.ac.at
SOURCE: J.Biochem.; (2001) 130, 3, 341-49 CODEN: JOBIAO ISSN: 0021-924X
DOCUMENT TYPE: Journal
LANGUAGE: English

L21 ANSWER 11 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2001:724811 SCISEARCH
THE GENUINE ARTICLE: 469LY
TITLE: Galactosyl transfer catalyzed by thermostable **beta -glycosidases** from Sulfolobus solfataricus and **Pyrococcus** furiosus: Kinetic studies of the reactions of galactosylated enzyme intermediates with a range of nucleophiles
AUTHOR: Petzelbauer I; Splechtna B; Nidetzky B (Reprint)
CORPORATE SOURCE: Univ Agr Sci, BOKU, Inst Food Technol, Div Biochem Engn, Muthgasse 18, A-1190 Vienna, Austria (Reprint); Univ Agr Sci, BOKU, Inst Food Technol, Div Biochem Engn, A-1190 Vienna, Austria
COUNTRY OF AUTHOR: Austria
SOURCE: JOURNAL OF BIOCHEMISTRY, (SEP 2001) Vol. 130, No. 3, pp. 341-349. Publisher: JAPANESE BIOCHEMICAL SOC, ISHIKAWA BLDG-3F, 25-16 HONGO-5-CHOME, BUNKYO-KU, TOKYO, 113, JAPAN. ISSN: 0021-924X.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 38
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L21 ANSWER 12 OF 26 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:125831 HCAPLUS
DOCUMENT NUMBER: 134:363126
TITLE: Characterization of β-glycosylhydrolases from **Pyrococcus** furiosus
AUTHOR(S): Kaper, Thijs; Verhees, Corne H.; Lebbink, Joyce H. G.;

Van Lieshout, Johan F. T.; Kluskens, Leon D.; Ward, Don E.; Kengen, Serve W. M.; Beerthuyzen, Marke M.; De Vos, Willem M.; Van der Oost, John

CORPORATE SOURCE: USA

SOURCE: Methods in Enzymology (2001), 330(Hyperthermophilic Enzymes, Part A), 329-346
CODEN: MENZAU; ISSN: 0076-6879

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 13 OF 26 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:627928 HCAPLUS

DOCUMENT NUMBER: 133:192395

TITLE: Method for enzymatically cleaving lactose, in particular, by using membrane diffusion reactors

INVENTOR(S): Novalic, Senad; Kulbe, Klaus Dieter

PATENT ASSIGNEE(S): Lactoprot Alpenlandische Milchindustrie und Handels-A.-G., Austria

SOURCE: PCT Int. Appl., 16 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000051441	A2	20000908	WO 2000-AT37	20000215
WO 2000051441	A3	20010301		
W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AT 9900370	A	20000215	AT 1999-370	19990304
EP 1158860	A2	20011205	EP 2000-904675	20000215
EP 1158860	B1	20020619		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AT 219331	E	20020715	AT 2000-904675	20000215
ES 2176166	T3	20021201	ES 2000-904675	20000215
AT 408447	B	20011126	AT 2000-239	20000217

PRIORITY APPLN. INFO.: AT 1999-370 A 19990304
WO 2000-AT37 W 20000215

L21 ANSWER 14 OF 26 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:121661 HCAPLUS

DOCUMENT NUMBER: 132:177431

TITLE: Preparation of a heat-resistant .**beta**.-**glycosidase** of **Pyrococcus** horikoshii and **cloning** of its encoding gene

INVENTOR(S): Matsui, Ikuo; Ishikawa, Kazuhiko; Ishida, Hiroyasu; Kosugi, Keiji

PATENT ASSIGNEE(S): Agency of Industrial Sciences and Technology, Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.
CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2000050870	A2	20000222	JP 1998-222866	19980806
JP 2995292	B2	19991227		
US 2002102635	A1	20020801	US 1999-369735	19990806

PRIORITY APPLN. INFO.: JP 1998-222866 A 19980806

L21 ANSWER 15 OF 26 MEDLINE on STN
ACCESSION NUMBER: 2000495120 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10931188
TITLE: Transgalactosylation by thermostable **beta-glycosidases** from **Pyrococcus** furiosus and Sulfolobus solfataricus. Binding interactions of nucleophiles with the galactosylated enzyme intermediate make major contributions to the formation of new beta-glycosides during lactose conversion.
AUTHOR: Petzelbauer I; Reiter A; Splechtna B; Kosma P; Nidetzky B
CORPORATE SOURCE: Division of Biochemical Engineering, Institute of Food Technology and Institute of Chemistry, Universitat fur Bodenkultur Wien, Vienna, Austria.
SOURCE: European journal of biochemistry / FEBS, (2000 Aug) 267 (16) 5055-66.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20001027
Last Updated on STN: 20001027
Entered Medline: 20001018

L21 ANSWER 16 OF 26 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:288276 BIOSIS
DOCUMENT NUMBER: PREV200000288276
TITLE: Comparative structural analysis and substrate specificity engineering of the hyperthermostable beta-glucosidase CelB from **Pyrococcus** furiosus.
AUTHOR(S): Kaper, Thijs [Reprint author]; Lebbink, Joyce H.G.; Pouwels, Jeroen; Kopp, Juergen; Schulz, Georg E.; van der Oost, John; de Vos, Willem M.
CORPORATE SOURCE: Laboratory of Microbiology, Department of Biomolecular Sciences, Wageningen University, Hesselink van Suchtelenweg 4, NL-6703 CT, Wageningen, Netherlands
SOURCE: Biochemistry, (May 2, 2000) Vol. 39, No. 17, pp. 4963-4970. print.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Jul 2000
Last Updated on STN: 7 Jan 2002

L21 ANSWER 17 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2001:177935 SCISEARCH
THE GENUINE ARTICLE: 403MB
TITLE: Kinetic study of a thermostable **beta-glycosidase** of Thermus thermophilus. Effects of temperature and glucose on hydrolysis and transglycosylation reactions
AUTHOR: Fourage L; Dion M; Colas B (Reprint)

CORPORATE SOURCE: Fac Sci & Tech, CNRS, FRE 2230, Unite Rech Biocatalyse, 2 Rue Houssiniere, BP 92208, F-44322 Nantes 3, France (Reprint); Fac Sci & Tech, CNRS, FRE 2230, Unite Rech Biocatalyse, F-44322 Nantes 3, France
COUNTRY OF AUTHOR: France
SOURCE: GLYCOCONJUGATE JOURNAL, (JUN 2000) Vol. 17, No. 6, pp. 377-383.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
ISSN: 0282-0080.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 37
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L21 ANSWER 18 OF 26 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2000141228 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10675537
TITLE: Novel substrate specificity of a membrane-bound **beta-glycosidase** from the hyperthermophilic archaeon **Pyrococcus** horikoshii.
AUTHOR: **Matsui I**; Sakai Y; Matsui E; Kikuchi H; Kawarabayasi Y; Honda K
CORPORATE SOURCE: National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki, Japan.. ikmatsui@nibh.go.jp
SOURCE: FEBS letters, (2000 Feb 11) 467 (2-3) 195-200.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000413
Last Updated on STN: 20000413
Entered Medline: 20000331

L21 ANSWER 19 OF 26 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2000-09594 BIOTECHDS
TITLE: Development of an ultra-high-temperature process for the enzymatic hydrolysis of lactose: II. Oligosaccharide formation by two thermostable **beta-glycosidases**;
Sulfolobus solfataricus and **Pyrococcus** furiosus **recombinant beta-glycosidase** production via vector-mediated gene transfer and **expression** in Escherichia coli
AUTHOR: Petzelbauer I; Zeleny R; Reiter A; Kulbe K D; *Nidetzky B
CORPORATE SOURCE: Univ.Vienna-Agr.Inst.Food-Technol.; Inst.Anim.Biotechnol.Tulln; Univ.Vienna-Agr.Inst.Chem.
LOCATION: Division of Biochemical Engineering, Institute of Food Technology, Universitat fur Bodenkultur Vienna (BOKU), Muthgasse 18, A-1190 Vienna, Austria.
Email: nide@edv2.boku.ac.at
SOURCE: Biotechnol.Bioeng.; (2000) 69, 2, 140-49
CODEN: BIBIAU
ISSN: 0006-3592
DOCUMENT TYPE: Journal
LANGUAGE: English

L21 ANSWER 20 OF 26 MEDLINE on STN
ACCESSION NUMBER: 1999326267 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10397869
TITLE: Development of an ultra-high-temperature process for the enzymatic hydrolysis of lactose. I. The properties of two

thermostable **beta-glycosidases**.
COMMENT: Erratum in: Biotechnol Bioeng 1999 Dec 20;65(6):following 676
AUTHOR: Petzelbauer I; Nidetzky B; Haltrich D; Kulbe K D
CORPORATE SOURCE: Division of Biochemical Engineering, Institute of Food Technology, Universitat fur Bodenkultur Wien (BOKU), Muthgasse 18, A-1190 Wien, Austria.
SOURCE: Biotechnology and bioengineering, (1999 Aug 5) 64 (3) 322-32.
Journal code: 7502021. ISSN: 0006-3592.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990925
Last Updated on STN: 20000327
Entered Medline: 19990913

L21 ANSWER 21 OF 26 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1999327626 EMBASE
TITLE: Gene analysis and enzymatic properties of thermostable . **beta**.-**glycosidase** from **Pyrococcus** kodakaraensis KOD1.
AUTHOR: Ezaki S.; Miyaoku K.; Nishi K.-I.; Tanaka T.; Fujiwara S.; Takagi M.; Atomi H.; Imanaka T.
CORPORATE SOURCE: T. Imanaka, Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
SOURCE: Journal of Bioscience and Bioengineering, (1999) 88/2 (130-135).
Refs: 22
ISSN: 1389-1723 CODEN: JBBIF6
COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

L21 ANSWER 22 OF 26 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1999:496230 SCISEARCH
THE GENUINE ARTICLE: 208XA
TITLE: **Cloning** and **expression** of a **beta-glycosidase** gene from Thermus thermophilus. Sequence and biochemical characterization of the encoded enzyme
AUTHOR: Dion M; Fourage L; Hallet J N; Colas B (Reprint)
CORPORATE SOURCE: UNIV NANTES, FAC SCI & TECH, UNITE RECH BIOCATALYSE, 2 RUE HOUSSINIERE, BP 92208, F-44322 NANTES 3, FRANCE (Reprint); UNIV NANTES, FAC SCI & TECH, UNITE RECH BIOCATALYSE, F-44322 NANTES 3, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: GLYCOCONJUGATE JOURNAL, (JAN 1999) Vol. 16, No. 1, pp. 27-37.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
ISSN: 0282-0080.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 67
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L21 ANSWER 23 OF 26 MEDLINE on STN
ACCESSION NUMBER: 1998058904 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9395451
TITLE: Molecular and biochemical characterization of an endo-beta-1,3- glucanase of the hyperthermophilic archaeon **Pyrococcus** furiosus.
AUTHOR: Gueguen Y; Voorhorst W G; van der Oost J; de Vos W M
CORPORATE SOURCE: Bacterial Genetics Group, Department of Microbiology, Wageningen Agricultural University, Hesselink van Suchtelenweg 4, NL-6703 CT Wageningen, The Netherlands.
SOURCE: Journal of biological chemistry, (1997 Dec 12) 272 (50) 31258-64.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF013169
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980129
Last Updated on STN: 19980129
Entered Medline: 19980115

L21 ANSWER 24 OF 26 MEDLINE on STN
ACCESSION NUMBER: 96394494 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8798600
TITLE: Comparison of a beta-glucosidase and a beta-mannosidase from the hyperthermophilic archaeon **Pyrococcus** furiosus. Purification, characterization, gene **cloning**, and sequence analysis.
AUTHOR: Bauer M W; Bylina E J; Swanson R V; Kelly R M
CORPORATE SOURCE: Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, USA.
SOURCE: Journal of biological chemistry, (1996 Sep 27) 271 (39) 23749-55.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U60214
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19980206
Entered Medline: 19961118

L21 ANSWER 25 OF 26 MEDLINE on STN
ACCESSION NUMBER: 96099293 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8522516
TITLE: Characterization of the celB gene coding for beta-glucosidase from the hyperthermophilic archaeon **Pyrococcus** furiosus and its **expression** and site-directed mutation in Escherichia coli.
AUTHOR: Voorhorst W G; Eggen R I; Luesink E J; de Vos W M
CORPORATE SOURCE: Department of Microbiology, Wageningen Agricultural University, The Netherlands.
SOURCE: Journal of bacteriology, (1995 Dec) 177 (24) 7105-11.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U37557; PIR-A27233; PIR-A28673; PIR-A29897;

```
                    PIR-B37168; SWISSPROT-P14288; SWISSPROT-P22498;
                    SWISSPROT-S03813
ENTRY MONTH:        199601
ENTRY DATE:         Entered STN: 19960219
                    Last Updated on STN: 19980206
                    Entered Medline: 19960122

L21   ANSWER 26 OF 26  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1994-13129  BIOTECHDS
TITLE:            Extremophiles in biotechnology;
                     production and application of e.g. thermophilic bacterium
                     and its enzyme (conference paper)
AUTHOR:           Rossi M; De Rosa M
CORPORATE SOURCE: CNR-Inst.Biochem.Protein-Enzymol.; Univ.Naples;
                  Univ.Naples-Inst.Biochem.Macromol.
LOCATION:         Istituto di Biochimica delle Proteine ed Enzimologie, CNR,
                  Via Marconi 10, 80125 Napoli, Italy.
SOURCE:           Prog.Biotechnol.; (1994) 9, Pt.1, 255-62
                  CODEN: PBITE3
DOCUMENT TYPE:    Journal
LANGUAGE:         English


=> d his

     (FILE 'HOME' ENTERED AT 10:21:50 ON 10 JUN 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:22:13 ON 10 JUN 2004
L1          1757 S BETA(W)GLYCOSIDASE?
L2         53153 S TETRAMER
L3            32 S L1 AND L2
L4             9 DUP REM L3 (23 DUPLICATES REMOVED)
L5          7080 S PYROCOCCUS
L6           156 S L1 AND L5
L7             8 S L2 AND L6
L8             3 DUP REM L7 (5 DUPLICATES REMOVED)
L9           741 S L5(A)HORIKOSHII
L10            6 S L1 AND L9
L11            2 DUP REM L10 (4 DUPLICATES REMOVED)
L12      6555393 S CLON? OR EXPRESS? OR RECOMBINANT
L13           75 S L6 AND L12
L14           26 DUP REM L13 (49 DUPLICATES REMOVED)
                 E KOSUGI Y/AU
L15          460 S E3
                 E ISHIDA H/AU
L16         6017 S E3
                 E ISHIKAWA K/AU
L17         8442 S E3
                 E MATSUI I/AU
L18          637 S E3
L19        15412 S L14 OR L15 OR L16 OR L17 OR L18
L20           29 S L1 AND L19
L21           26 DUP REM L20 (3 DUPLICATES REMOVED)

=> s l21 and l5
L22           26 L21 AND L5

=> s l9 and l22
L23            2 L9 AND L22

=> d 1-2 ibib ab

L23  ANSWER 1 OF 2     MEDLINE on STN
```

ACCESSION NUMBER: 2000141228 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10675537
TITLE: Novel substrate specificity of a membrane-bound **beta-glycosidase** from the hyperthermophilic archaeon **Pyrococcus horikoshii**.
AUTHOR: **Matsui I**; Sakai Y; Matsui E; Kikuchi H; Kawarabayasi Y; Honda K
CORPORATE SOURCE: National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki, Japan.. ikmatsui@nibh.go.jp
SOURCE: FEBS letters, (2000 Feb 11) 467 (2-3) 195-200. Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000413
Last Updated on STN: 20000413
Entered Medline: 20000331

AB A **beta-glycosidase** gene homolog of **Pyrococcus horikoshii** (BGPh) was successfully **expressed** in Escherichia coli. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85 degrees C for 15 min. The optimum pH was 6.0 and the optimum temperature was over 100 degrees C, respectively. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90 degrees C (pH 6.0) was 15 h. BGPh has a novel substrate specificity with k(cat)/K(m) values high enough for hydrolysis of beta-D-Glcp derivatives with long alkyl chain at the reducing end and low enough for the hydrolysis of beta-linked glucose dimer more hydrophilic than aryl- or alkyl-beta-D-Glcp.

L23 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:121661 HCAPLUS
DOCUMENT NUMBER: 132:177431
TITLE: Preparation of a heat-resistant .**beta**.-**glycosidase** of **Pyrococcus horikoshii** and **cloning** of its encoding gene
INVENTOR(S): Matsui, Ikuo; Ishikawa, Kazuhiko; Ishida, Hiroyasu; Kosugi, Keiji
PATENT ASSIGNEE(S): Agency of Industrial Sciences and Technology, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2000050870	A2	20000222	JP 1998-222866	19980806
JP 2995292	B2	19991227		
US 2002102635	A1	20020801	US 1999-369735	19990806

PRIORITY APPLN. INFO.: JP 1998-222866 A 19980806

AB The gene encoding a novel heat-resistant .**beta**.-**glycosidase** is isolated from **Pyrococcus horikoshii** strain JCM9974. The .**beta**.-**glycosidase** prepared from transgenic Escherichia coli exhibits a temperature optimum >100° and a pH optimum 6.0.

=> d his

(FILE 'HOME' ENTERED AT 10:21:50 ON 10 JUN 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:22:13 ON 10 JUN 2004

L1	1757	S	BETA(W)GLYCOSIDASE?
L2	53153	S	TETRAMER
L3	32	S	L1 AND L2
L4	9	DUP REM	L3 (23 DUPLICATES REMOVED)
L5	7080	S	PYROCOCCUS
L6	156	S	L1 AND L5
L7	8	S	L2 AND L6
L8	3	DUP REM	L7 (5 DUPLICATES REMOVED)
L9	741	S	L5(A)HORIKOSHII
L10	6	S	L1 AND L9
L11	2	DUP REM	L10 (4 DUPLICATES REMOVED)
L12	6555393	S	CLON? OR EXPRESS? OR RECOMBINANT
L13	75	S	L6 AND L12
L14	26	DUP REM	L13 (49 DUPLICATES REMOVED)
		E	KOSUGI Y/AU
L15	460	S	E3
		E	ISHIDA H/AU
L16	6017	S	E3
		E	ISHIKAWA K/AU
L17	8442	S	E3
		E	MATSUI I/AU
L18	637	S	E3
L19	15412	S	L14 OR L15 OR L16 OR L17 OR L18
L20	29	S	L1 AND L19
L21	26	DUP REM	L20 (3 DUPLICATES REMOVED)
L22	26	S	L21 AND L5
L23	2	S	L9 AND L22

	Issue Date	Pages	Document ID	Title
1	20020801	26	US 20020102635 A1	METHODS FOR MAKING AND USING A THERMOPHILIC ENZYME AS A BETA-GLYCOSIDASE
2	20030114	40	US 6506592 B1	Hyperthermophilic alpha-glucosidase gene and its use
3	20000620	10	US 6077695 A	Method of producing derivatives of Glc-.beta. 1-4Glc-N-acetyl

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1	20020801	26	US 20020102635 A1

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1	20020801	26	US 20020102635 A1	METHODS FOR MAKING AND USING A THERMOPHILIC ENZYME AS A BETA-GLYCOSIDASE
2	19991109	20	US 5981835 A	Transgenic plants as an alternative source of lignocellulosic-degrading enzymes
3	19901030	15	US 4966856 A	Analytical element and the analytical method using the element
4	19890919	22	US 4868106 A	Analytical element and method for determining a component in a test sample

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5	19870728	17	US 4683198 A	Novel maltose dehydrogenase, process for its production, and analytical method using the same

	L #	Hits	Search Text
1	L1	102	beta adj glycosidas?
2	L2	7459	tetramer
3	L3	0	l1 same l2
4	L4	91036	clon? or express? or recombinant
5	L5	3	l1 same l4
6	L6	864	pyrococcus
7	L7	1	l1 same l6
8	L8	49485	MATSUI ISHIKAWA ISHIDA KOSUGI
9	L9	5	l1 and l8